# The Nile Crocodile of the Okavango Delta in Health and Disease

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# DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis
is my own original work
and that I have not previously in its entirety or in part
submitted it at any university for a degree.

**Date:** .....8/11/2006.....

## ABSTRACT

Crocodile farming has become an important industry in Southern Africa over the last three decades. The diseases occurring in farmed crocodiles have been well researched, which has contributed to the success of modern crocodile farming operations. However, very little research has been done on diseases in wild crocodiles, and the normal physiology and disease prevalence of wild crocodiles remains largely unknown.

In this study Nile crocodiles were captured in the Okavango Delta, Botswana. Blood was collected and normal haematological and blood biochemical ranges were established for a sub-sample of the population (haematology n=38, biochemistry n=35). The ranges obtained were generally in line with those reported for other species and farmed Nile crocodiles, except for mean haematocrit and total protein, which were relatively low. Parameters were also compared between males and females, as well as between size classes. Females had significantly greater mean red cell count, eosinophils, total protein and potassium than males. Subadults had significantly greater mean haematocrit, haemoglobin, eosinophils, basophils, total protein, globulin, sodium and potassium than yearlings and juveniles. Yearlings had significantly higher blood glucose than juveniles.

Cloacal swabs were collected (n=29), which were cultured to establish the normal intestinal flora of these crocodiles. The intestinal flora was found to be diverse, with a mean of 2.7 bacterial species per crocodile. No Salmonella were cultured. Approximately half the crocodiles (48.3 %) also had a fungal component to their intestinal flora. A probiotic was produced based on the normal intestinal flora of the wild crocodiles. The potential for this probiotic to reduce mortalities and improve growth in farmed hatchlings was tested in a controlled experiment. No significant beneficial effect was obtained.

A disease survey was carried out on the wild crocodiles by (i) a general clinical examination (n=144), (ii) serological testing for mycoplasmosis (n=30), and (iii) bloodsmear examination for blood parasites (n=38). No clinically apparent sick crocodiles were observed. No antibodies to *Mycoplasma crocodyli* were detected. The prevalence of hepatozoonosis was 55.3 %. There was no significant difference in the haematological parameters of *Hepatozoon*-infected and un-infected crocodiles.

## OPSOMMING

Oor die afgelope drie dekades het krokodil boerdery in Suiderlike Afrika gegroei tot 'n groot industrie. Baie navorsing is al op die siektes van krokodille onder boerdery omstandighede gedoen, wat tot die sukses van moderne krokodil boerdery bygedra het. Baie min navorsing is al oor die siektes van wilde krokodille gedoen, en die siektes en normale fisiologie van wilde Nyl krokodille bly hoofsaaklik onbekend.

In hierdie studie was Nyl krokodille in die Okavango Delta, Botswana, gevang. Bloed was getrek en die normale waardes vir 'n sub-seksie van die bevolking se hematolgie (n=38) en bloed biochemie (n=35) was vasgestel. Die waardes was vergelykbaar met die van Nyl krokodille onder boerdery omstandighede, sowel as waardes wat al van ander spesies geraporteer is, behalwe vir gemiddelde hematokrit en totale proteïne, wat laag was. Parameters was tussen mannetjies en wyfies sowel as tussen verskillende groottes vergelyk. Wyfies het merkwaardig hoër gemiddelde rooiseltelling, eosinofiele, totale proteïn, en kalium as mannetjies gehad. Sub-volwassenes het merkwaardig hoër gemiddelde hematokrit, hemoglobin, eosinofiele, basofiele, totale proteïn, globulien, natrium en kalium as jaar-oud en jong krokodille gehad. Jaar-oud krokodille het merkwaardige hoër bloed glukose as jong krokodille gehad.

Kloak deppers was versamel (n=29), en kwekings gedoen om die normale dermkanaal flora vas te stel. Daar was 'n breë spektrum van dermkanaal flora, met 'n gemiddeld van 2.7 bakterie spesies per krokodil. Geen Salmonella was geïsoleer nie. Daar was een of meer fungi van 48.3 % van die deppers geïsloeer. 'n Probiotikum was gemaak, gebaseer op die normale dermkanaal flora van die wilde krokodille. Die vermoë van die probiotikum om sterftes te verminder, en groei te verbeter, was op klein krokodile onder intensiewe omstandighede getoets. Daar was geen voordeel daarin gevind.

'n Siekte opname was op wilde krokodille gedoen deur (i) 'n algemene kliniese ondersoek (n=144), (ii) serologiese toetse vir mikoplasmose (n=30), en (iii) bloedsmeer ondersoek vir bloedparasite (n=38). Geen ooglopend siek krokodille was tydens bemonstering gevind, en daar is geen teenliggame teen *Mycoplasma crocodyli* gevind. Die voorkoms van *Hepatozoon pettiti* was 55.3 %. Daar was geen betekenisvolle verskil tussen die hematologie van die *Hepatozoon*-besmette en nie-besmette krokodille nie.

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# CHAPTER 1

## Captive and Wild crocodilian diseases

### Introduction

Research on crocodilian biology began in the 1800's, however research on crocodilian diseases only began in earnest in the 1980's, as the crocodile ranching/farming industry expanded. Crocodiles are very sensitive to stress, and a high incidence of disease and mortalities were experienced initially due to poor husbandry practices (Foggin, 1992a). This situation provided the opportunity to investigate the diseases occurring in farmed crocodiles. However, very little research on the health status of wild Nile crocodiles has been undertaken.

### Review

### Crocodilian Diseases

Diseases occurring in farmed crocodilians have been well documented over the past two decades. There are also a limited number of reports of diseases in wild crocodilians. The more important diseases are briefly reviewed here.

### Viral Diseases

Pox

Infection with a Parapoxvirus was first reported in Caimans (*Caiman crocodylus*) in the USA (Jacobsen et al., 1979). The disease is characterized by grayish white crusty lesions on the oral mucosa and skin.

Crocodile pox in the Nile crocodile (*Crocodylus niloticus)* has since been reported by a number of researchers (Foggin, 1987; Horner, 1988; Pandey et al., 1990; Huchzermeyer et al., 1991). It is characterized by brown crusty lesions on the skin. It has only been reported in farmed crocodiles up to 12 months of age, and appears to be stress associated. Morbidity in farmed crocodiles can be high, but the mortality is usually low. The genome of the crocodile pox virus from Nile crocodiles in Zimbabwe was sequenced by Afonso et al. (2006). More severe erosive skin lesions have been shown to be caused by an atypical pox virus (Huchzermeyer et al., 2006). Clinically healthy carriers can shed the pox virus, and it is presumed that wild crocodiles are a reservoir of infection. The exact mode of

transmission is unknown, but is likely to be through contaminated water (Huchzermeyer, 2003).

Adenovirus

Adenovirus infection was first recorded in hatchling *C. niloticus* in Zimbabwe (Jacobson et al., 1984), and is now a common problem on Zimbabwean crocodile farms (Foggin, 1992b). Hatchlings under 5 months of age become infected by horizontal transmission, although vertical transmission has also been demonstrated. The virus causes a fatal hepatitis, with distinct intranuclear inclusion bodies in hepatocytes. Symptomatically there is little to be seen. There is also a chronic form, and as such it is a major cause of runting.

West Nile Virus (WNV)

This virus has caused outbreaks of disease with high mortality in farmed American alligators (*Alligator mississippiensis*) in Florida. (Miller et al., 2003). In a serological survey in Israel the seroprevalence rate was 70 % in farmed Nile crocodiles tested (Steinmann et al., 2003), and Farfan-Ale et al. (2006) found a seroprevalence of 86 % in farmed crocodiles in Mexico. Crocodilians may play an important epidemiological role as natural reservoirs of the virus.

Newcastle disease (Paramyxovirus)

Seropositive farmed Nile crocodiles have been documented (Thomson, 1972).

**Bacterial Diseases**

Chlamydiosis

Chlamydiosis is an important disease in young farmed Nile crocodiles (Huchzermeyer et al., 1994). In the acute form it causes a severe hepatitis, resulting in acute death. In the chronic form there is bilateral blepharo-conjunctivitis with eventual blindness (Huchzermeyer, 2003). Chlamydia has caused very high mortality in Indo-Pacific crocodiles, *Crocodylus porosus* (Huchzermeyer et al., 2006). *Chlamydia psittaci* has been identified in the African clawed frog, *Xenopus laevis* (Newcomer et al., 1982), and it is believed that wild crocodiles may be a natural reservoir.

## Mycoplasmosis

A mycoplasma species was first found to cause polyarthritis with some lung involvement in farmed Nile crocodiles in Zimbabwe. The animals displayed progressive lameness and paresis (Mohan et al., 1995). Kirchoff et al. (1997) assigned this mycoplasma to a new species: *Mycoplasma crocodyli*. This disease results in major losses on some farms. In a recent outbreak in Zimbabwe, morbidity was over 50 % and mortality over 20 %. A vaccine was made to help control this outbreak (Mohan et al., 2001). At least 2 highly pathogenic new local species occur in SA, and mycoplasmosis has become an important disease on Southern African crocodile farms (Picard, pers. comm.).

*Mycoplasma alligatoris* causes a similar disease in American alligators, characterized by pneumonia, pericarditis and arthritis (Brown et al., 1996 and 2001; Clippinger et al., 2000). Its ability to infect other crocodilian species has been demonstrated with broad-nosed caimans (*Caiman latirostris*) (Pye et al., 2001). However, the epidemiology of mycoplasmosis remains unresolved. Huchzermeyer (2003) suggested that wild crocodiles may act as reservoirs of infection. This theory is supported by a recent seroprevalence study which found 5.4 % of wild American alligators positive for *M. alligatoris* antibodies (Brown et al., 2005).

## Salmonellosis

Farmed crocodiles frequently harbour a wide range of *Salmonella* serovars as part of their normal intestinal flora (Scott and Foster, 1997; Manolis et al., 1991; Obwolo and Zwart, 1993). Wild African dwarf crocodiles (*Osteolaemus tetraspis)* and wild Nile crocodiles have also been found to carry *Salmonella* (Huchzermeyer et al., 2000; Madsen et al., 1998). Nevertheless, *Salmonella* is the most frequently implicated bacterium in mortalities caused by bacterial infection (Foggin, 1992b). Salmonellosis manifests itself in farmed crocodiles as either septicaemia or enteritis. Outbreaks on crocodile farms have caused severe losses (Huchzermeyer, 1991). Stress, unhygienic conditions and feeding of infected carcasses are important factors in the epidemiology of the disease in farmed crocodiles. Not only is *Salmonella* important as a cause of death in farmed crocodiles, it also has zoonotic potential. Some of the serotypes isolated from crocodiles are pathogenic in humans. Manolis et al. (1991) reported 16 % prevalence of *Salmonella* on *C. porosus* and *C. johnstoni* carcasses destined for human consumption, despite proper slaughter procedure, while Madsen, (1993 and 1996) found *Salmonella* in up to 33 % of meat samples from farmed Nile crocodiles.

A large variety of other bacteria cause septicaemia in farmed crocodiles (Foggin, 1987; Foggin, 1992b; Buenviaje et al., 1994; Ladds and Simms, 1990). Bacterial infections are promoted by low environmental temperatures, inappropriate diet, poor hygiene and stress (Trutnau and Sommerlad, 2006). Often bacteria causing septicaemia are part of the normal gastrointestinal flora. Currently the full range of normal intestinal flora in wild Nile crocodiles is unknown.

Dermatophilosis (Brown Spot Disease)

A *Dermatophilus sp.* is a common cause of skin disease in *C. porosus* and *C. johnstoni* in Australia (Buenviaje et al., 1997). Brown spot disease is characterized by brown spots, ulcerations, erosions and granulomas. It has not been recorded in Nile crocodiles.

**Fungal Diseases**

The range of fungi that have been isolated from farmed and wild crocodilians was reviewed by Huchzermeyer (2003). Most are part of the normal intestinal flora, and are opportunistic pathogens. Stress and unfavourable environmental conditions are important factors. Infections may be superficial, organ specific, or generalized.

**Parasites**

**Protozoa**

Coccidia described in crocodiles are usually non-pathogenic, although coccidiosis can be an important disease in farmed hatchlings. The parasite is found mainly in the intestinal mucosa, where it causes fibrinous enteritis. Shizonts and sporocysts can be found in the organs (Foggin, 1992b).

Several *Hepatozoon* species have been described in crocodilians. Gametocytes are found in erythrocytes, or free in the blood. The intermediate hosts are usually hematophagous insects or possibly leeches (Khan et al., 1980; Lainson et al., 2003). While developmental stages have been found in leeches, actual transmission of Hepatozoon spp. by leeches has not been proven. In Nile crocodiles, *Hepatozoon pettiti* was first described by Thiroux (1910) from Senegal and Uganda. Then *H. sheppardi* was described from Nile crocodiles in Mozambique (Santos Dias, 1952). Gomersall et al. (2006) recently reported a 32.8 % prevalence of *H. pettiti* in Okavango Nile crocodiles. *Hepatozoon* infection in crocodiles appears to be non-pathogenic.

A *Giardia* species has been found in farmed Nile crocodiles in South Africa (Huchzermeyer, 2003).

*Trypanosoma grayi* was described by Hoare (1929) in Nile crocodiles. There is a very low level of parasitaemia and it does not appear to be pathogenic.

**Helminths**

Ascaridoids: A large number of species have been described from the Nile crocodile, where they occur in the stomach, often in association with gastric ulcers. Ascaridoids are, for the most part, non-pathogenic. Recently the ascaridoid nematodes *Dujardinascaris madagascariensis, Dujardinascaris dujardini, Gedoelstascaris vandenbrandeni* and *Multicaecum agile* were recovered from the stomach contents of wild Nile crocodiles from the Okavango River (Junker et al., 2006).

*Paratrichosoma* sp. is found in zigzagging burrows in the ventral skin of many crocodile species, including wild *C. niloticus* in Lake Kariba (Foggin, pers. comm.).

*Trichinella* were not known to occur in reptiles until 1995, when *Trichinella sp* was detected in 40 % of farm raised Nile crocodiles in Zimbabwe (Foggin et al., 1997). This was found to be a new species, *T. zimbabwensis* (Pozio et al., 2002). This has important zoonotic potential.

Trematodes: a large number of species have been reported, both from the intestinal tract and the kidneys.

Filarial worms also occur.

Cestodes: have not been found in crocodiles.

Pentastomes of six genera occur in 14 of the 23 crocodilian species. The Nile crocodile is host to six species of the genera *Sebekia, Alofia and Leiperia* (Junker and Boomker, 2006). They occur in the respiratory tract of the crocodile, where they may invoke granulomatous pneumonia. They utilize fish as their intermediate hosts.

**Crocodilian Haematology and Blood Chemistry**

Reptilian clinical pathology provides some special challenges (Wilkinson, 2004; Campbell, 1996). Several non-pathological factors can affect clinicopathological parameters: site of blood collection (degree of lymph contamination is influenced by site), temperature, season, gender and stress. Because reptiles maintain a less precise homeostasis than mammals and birds, blood biochemical "normal ranges" are wider.

The presence of nucleated erythrocytes and thrombocytes precludes the use of automated cell counters. Leukocyte counts must be performed manually. Manual counts are imprecise with a coefficient of variation of 30 % (Heard et al., 2004). Identification of cells is a problem due to confusing morphology and lack of standardized nomenclature for leukocytes.

Certain haematological values have been reported from farmed Nile crocodiles (Makinde and Alemu, 1991; Foggin, 1987; Thurman, 1990). Data obtained from farmed crocodiles may not represent true normal values, due to their stressed metabolic state. Watson (1990) also reported limited haematological values from a small group of captive Nile crocodiles (n=5). The mean leukocyte count of $1.5x10^3/\mu l$ was much lower than that found by Makinde and colleagues in farmed crocodiles ($6.4x10^3/\mu l$), and the differential count also differed vastly. This may have been due to Watson's small sample size, or may reflect a true variation between populations. Some haematological values have been reported for other species (Carmena-Suero et al., 1979; Canfield, 1985; Millan et al., 1997; Glassman et al., 1981; Stacey and Whitaker, 2000; Turton et al., 1997; Troiano et al., 1996; Mateo et al., 1984; Barnett et al., 1998 and 1999) and are summarized in Table 2.9.

Blood biochemical values reported for farmed Nile crocodiles (Foggin, 1987; Thurman, 1990) are similar to the limited values available from wild specimens (Swannepoel et al., 2000; Leslie, 1997). Values reported from other crocodile species (Millan et al., 1997; Stacey and Whitaker, 2000; Siruntawineti and Ratanakorn, 1994; Troiano and Althaus, 1993; Sigler, 1991; Tourn et al., 1994) show a reasonably wide range across the species (Table 2.11). Barnett et al. (1999) compared haematology and certain blood chemistry values of captive and wild American alligators, and found the wild alligators to be relatively anaemic.

**Crocodilian Normal Intestinal Flora**

Huchzermeyer et al. (2000) reported the intestinal flora isolated from wild-caught African dwarf crocodiles (*O. tetraspis*). A wide range of bacterial and fungal species were isolated, with a range of 1-5 bacteria and 0-5 fungal species per crocodile. Salmonellae isolated from wild Nile crocodiles from Lake Kariba (Madsen et al., 1998), and from wild American alligators (Scott and Foster, 1997) have also been documented.

Other studies have dealt with captive crocodilians. Misra et al. (1993) reported the normal flora of captive gharials (*Gavialis gangeticus*). Prevalence of salmonellae has been studied in healthy captive crocodilians (Obwolo and Zwart, 1993; Manolis et al., 1991).
The normal intestinal flora of wild Nile crocodiles therefore remains largely unknown, as does the difference between farmed and wild specimens.

**Objectives**

Crocodilians are terminal predators, and as such are good indicators of the state of their environment (Best, 1973; Vermeer et al., 1974; Matthiessen et al., 1982; Delany et al., 1988; Phelps et al., 1989; Jennings et al., 1989; Woodward et al., 1993; Guillette et al., 1994; Guillette et al., 1996; Crain and Guillette, 1998; and Crain et al., 1998). Consequently crocodilians are ideal species to study to assess the ecology of a system. As so little is known about the physiology and diseases of wild crocodiles at this stage, a certain amount of baseline data is essential for future comparisons.

Therefore the objectives of my study are:

1. To establish normal ranges for haematology and blood biochemistry in wild Nile crocodiles.
2. To establish the normal range of intestinal flora in wild Nile crocodiles.
3. Develop a probiotic based on the normal intestinal flora and test the application of this in farmed hatchlings.
4. Conduct a disease survey on the Nile crocodile population in the Okavango region to obtain baseline information on disease prevalence.

Not only will this study contribute baseline information about wild Nile crocodiles, it will also be of significance to crocodile farmers. The crocodile farming industry has grown into a massive business internationally (Macgregor, 2002). Increased knowledge and improved husbandry practices have resulted in a much lower hatchling mortality rate in recent years. Nevertheless many of the diseases do still occur on crocodile farms, and occasionally result in severe financial loss. Examples include Salmonellosis (Huchzermeyer, 1991), bacterial septicaemias (Foggin, 1992b), Mycoplasmosis (Mohan et al., 2001), Chlamydiosis (Huchzermeyer, 2006) and West Nile virus (Miller et al., 2003). A greater knowledge of physiology of wild crocodiles may help us better understand the host-pathogen-environment interactions to the benefit of the farming industry. Knowledge of the

prevalence of diseases in wild crocodiles will contribute to the understanding of the epidemiology of these diseases. A good understanding of the epidemiology of a disease is vital in order to develop control strategies for that disease on a crocodile farm.

One of the most common diseases among farmed crocodiles is enteritis. Frequently this progresses to a bacterial septicaemia, one of the main causes of death in farmed crocodilians (Foggin, 1992a; Foggin, 1992b; Buenviaje et al., 1994). Because of their unnatural environment and diet, farmed hatchlings often fail to develop a normal mixed intestinal flora (Huchzermeyer, 2003). In other species the rapid establishment of bacterial communities in the intestinal tract is thought to be essential for the prevention of colonization by pathogenic bacteria (Nava et al., 2005). The development of a probiotic appropriate for use in crocodiles has the potential to reduce the incidence of enteritis and septicaemia. Reducing mortality rate in hatchlings will have a big influence on farm profitability.

Veterinary involvement on crocodile farms remains important to limit production loss due to disease. To date, diagnostics have relied primarily on post mortem examinations in the face of a disease outbreak. In veterinary medicine, clinical pathology is widely employed as a diagnostic tool in many species. Millan et al. (2000) demonstrated the application of reference ranges for *C. porosus* as a basis in disease investigations, and Glassman et al. (1981) demonstrated predictable changes to the heamograms of American alligators infected with *Aeromonas hydrophila*. However, the usefulness of clinical pathology in Nile crocodiles is restricted by the lack of reference ranges for haematological and biochemical values. The limited studies to date have been on farmed Nile crocodiles, which due to their stressed metabolic state, do not necessarily represent true normal values.

The objective to establish normal haematological and biochemical values for wild Nile crocodiles, will contribute towards reference ranges for the species. Such ranges will make the interpretation of laboratory results easier, making clinical pathology a viable diagnostic tool for use in Nile crocodiles in both the zoo and crocodile farm environment.

**Study site**

The Okavango Delta, the world's biggest RAMSAR site, is a huge wetland within the Kalahari desert, and is situated in north west Botswana (Figure 1.1).

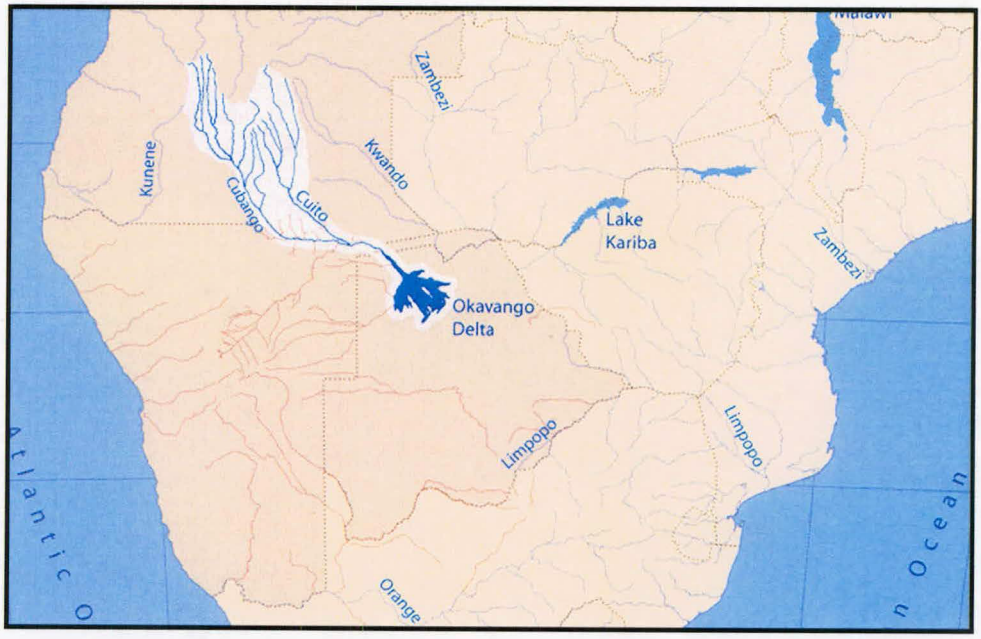


Figure 1.1: The geographical position of the Okavango Delta in Botswana, and its tributaries.

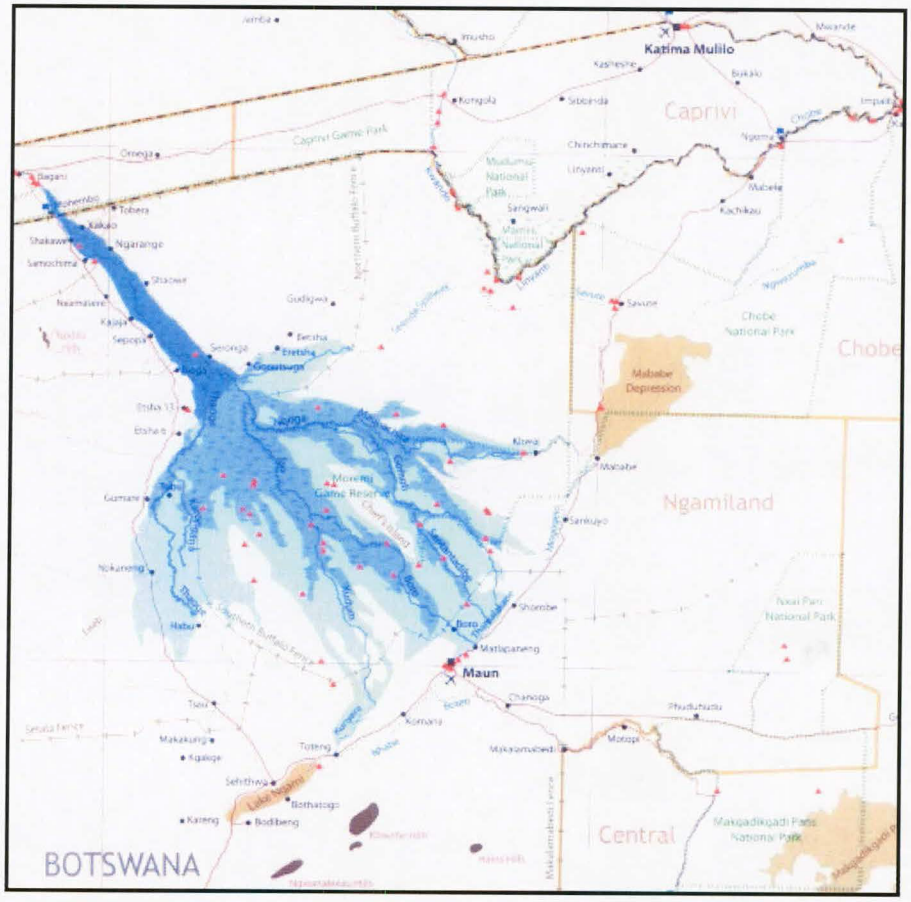


Figure 1.2: Map of the Panhandle region and of the Delta itself.

It covers an area of approximately 16 000 $km^2$ in the dry season, increasing to over 22 000 $km^2$ with the annual flood. The 111 250 $km^2$ active catchment area falls entirely within Angola, where the two main tributaries, the Cubango and Cuito jointly deliver an annual average of 9.38 $km^3$ of water to the Okavango. Due to the geology of the catchment, this incoming water is low in nutrients and sediment. About another 3.2 $km^3$ of water is added to the system through rain falling directly on the Delta (Mendelsohn and Obeid, 2004). The Okavango River flows through Namibia briefly before entering Botswana and forming a broad floodplain, known as the Panhandle (Figure 1.2).

An estimated 40 % of incoming water leaks into the surrounding swamps by the time the river leaves the Panhandle. The remaining 60 % is distributed down three main channels, which fan out to form the Delta. The Okavango Delta consists of permanent and seasonal swamp, which is inundated during the annual flood. The size and timing of the annual flood depends more on rainfall in the catchments area as opposed to local rainfall. In the panhandle region the flood peaks between February and April reaching the town of Maun (in the south) between June and August.

The northern part of the Delta is characterized by shallow water, flooded grasslands, backwater swamps, ox-bow lakes and hidden lagoons mostly interconnected by narrow waterways. Only a few main channels lined by tall reeds (mainly *Phragmites australis*), carry the remainder of the Okavango's water southwards through the Delta. The permanent and seasonal swamp, together form a unique ecosystem, providing high quality habitat for a great many species. As a keystone species, the Nile crocodile helps maintain the fragile balance within this ecosystem. Crocodiles are unevenly distributed throughout the Delta, with the majority of the breeding population occurring in the 120 km long Panhandle. During the height of the flooding season, crocodiles disperse into the floodplains, probably to follow the fish and find dry ground for basking during the cool winter months.

The climate of the area is sub-tropical with hot, rainy summers and cool, sunny and dry winters. Average daily maximum temperatures range from 35 °C in October to 26 °C in July. Average daily minimum temperature falls to 7 °C in the coldest months of June and July. The rainy season starts in November and ends in February.

Fauna and flora

There are over 80 fish species in the Delta. The system supports between 100 and 200 kilograms of fish per hectare. This is much lower than many other fresh water systems, primarily due to the water's low level of nutrients (Mendelsohn and Obeid, 2004).

Invertebrates are abundant. Biodiversity is lowest in the main flowing channels, slightly higher in the vegetated side channels, and higher still in quiet vegetated backwaters and lagoons.

The number of large mammals in the Delta varies with season between approximately 160 000 and 260 000. Due to the influence of man in the panhandle region, game concentrations there are low, and the diversity restricted to the Nile crocodile, hippopotamus (*Hippopotamus amphibious*), elephant *(Loxodonta africana*), lechwe (*Kobus lechwe*), two types of large monitor lizard (*Varanus niloticus* and *V. albigularis*), leopard (*Panthera pardus*), common bushbuck (*Tragelaphus scriptus*) and sitatunga (*Tragelaphus spekei*).

The vegetation of the panhandle region is dominated by the grasses *Vossia cupidata* (hippo grass) and *Echinochloa* (Limpopo grass), the sedge *Cyperus papyrus* (papyrus), rushes *Typha capensis* (bullrush) and reeds *Phragmites mauritianus* (phragmites).

**Study Animal**

The crocodilians of today are the only living representatives of the Archosauria or "ruling reptiles", one of the most successful groups of vertebrates ever known. This group included the dinosaurs and pterosaurs.

The history of the crocodilians has been reviewed by Bellairs (1987) and Buffetaut (1979 and 1989). Early crocodilians appeared in the Upper Triassic, some 200 million years ago. These are placed in the order Protosuchia, and were lizard-like creatures less than one meter in length, with a broad head and relatively short narrow snout, and long legs. During the Mesozoic (230 – 65 million years ago) crocodilians radiated into a great variety of broad and narrow snouted types. Modern crocodilians appeared and became dominant some 80 million years ago. Todays crocodilians are classified in the suborder Eusuchia, and family Crocodylidae. The twenty-three living species are grouped into three subfamilies: the Crocodylinae, the Alligatorinae and the Gavialinae (Ross and Magnusson, 1989). The distinction between crocodilians is based mainly on anatomical differences of the skull and scale patterns of the skin. The present range of crocodilians is limited to the tropical and subtropical regions of the world.

The Nile crocodile is the most widespread and abundant of the three crocodile species that occur in Africa. It occurs throughout the continent south of the Sahara, in a variety of wetland habitats, including coastal areas (Taplin and Loveridge, 1988). Historically its

distribution in southern Africa extended down the east coast as far as the Kei River (Figure 1.3).

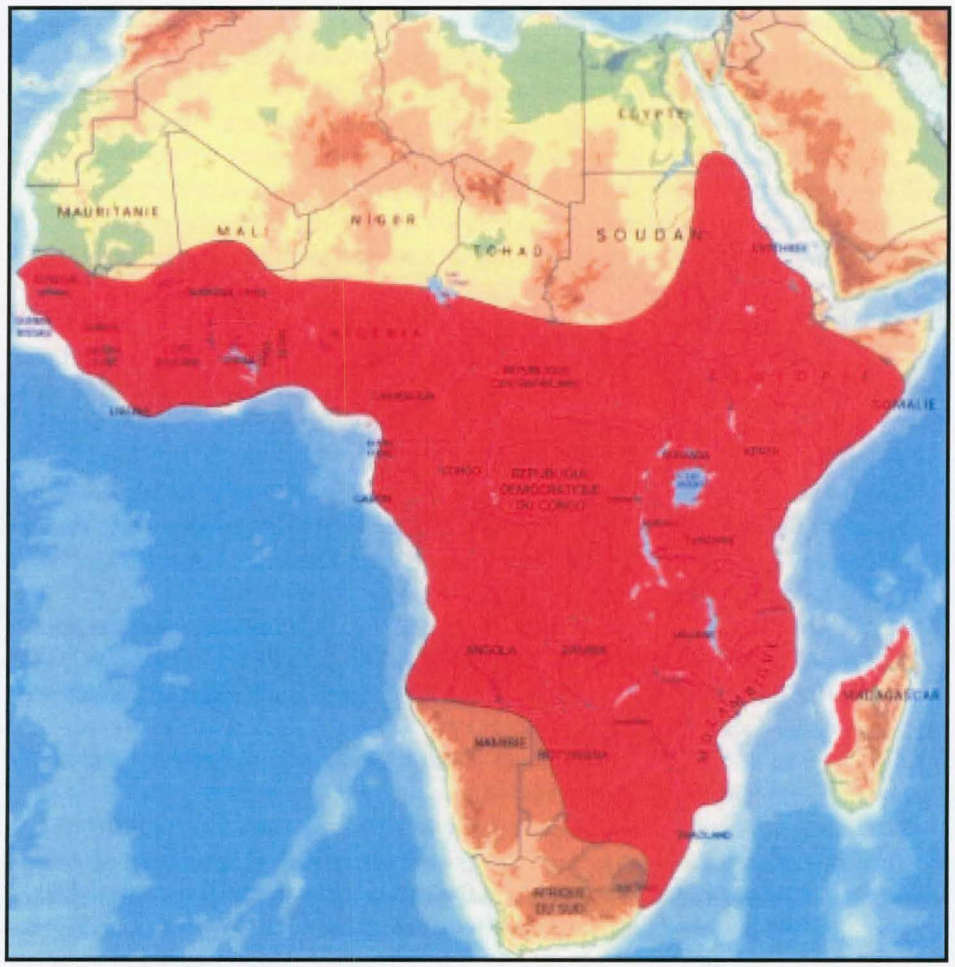


Figure 1.3: The distribution of the Nile crocodile in Africa and Madagascar. (Adapted from Trutnau and Sommerlad, 2006).

The Nile crocodile is dark olive to brown, with a light yellow abdomen. Being reptiles, they are ectothermic and regulate their body temperature behaviourally, by moving between sun-exposed sandbanks and the water. Typical adult lengths are around 3.5 m, but they can grow up to 5 m (Groombridge, 1987). Sexual maturity is reached from 2.9 m total length for males, and 2.2 m for females (Cott, 1961). Nesting occurs in a hole in the ground, where an average of 50 eggs are laid. Nile crocodiles exhibit temperature-dependent sex determination (Leslie, 1997). Hatchlings emerge after an incubation period of approximately 90 days, in early to mid summer, and parental protection occurs (Pooley and Gans, 1976; Pooley, 1977). There is a very high mortality rate in their first year of life due to predation.

The diet of the Nile crocodile changes ontogenetically, consisting predominantly of insects and crustaceans in young crocodiles, while fish forms a major part of the diet in crocodiles over one metre (Cott, 1954). Adult crocodiles are often the largest predators in their aquatic environments, with terrestrial mammals including humans and livestock falling victim to them.

The Nile crocodile was heavily exploited throughout Africa during the 1950's and 1960's (Parker and Watson, 1970). Legal protection in the 1970's (through a CITES Appendix 1 listing in 1973) resulted in significant recoveries of crocodile populations in several countries, including in the Okavango Delta. In Botswana today, Nile crocodiles are found in the Okavango Delta and the Kwando/Linyanti/Chobe Rivers.

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# CHAPTER 2

## Normal Haematology and Blood Biochemistry of Wild Nile Crocodiles (*Crocodylus niloticus*) in the Okavango Delta, Botswana.

### Introduction

Crocodile farming has developed into a large global industry over the past 25 years. According to Luxmore (1992) there were 597 commercial farms, with a total stock of 1.1 million animals. The biggest crocodile farm is in China and houses about 18 000 crocodiles, while the Sumutprakarn Farm in Thailand has 3 700 breeding stock (Trutnau and Sommerlad, 2006).

In a comprehensive report on the international trade in crocodilian skins, MacGregor (2002) states that since the promulgation of CITES, the proportion of crocodilian skins supplied to the industry from wild harvests has diminished dramatically, from over 99 % in 1983 to only 6 % in 1999. Of an estimated total of 1 182 469 skins, 70 381 originated from wild harvest, 255 945 from ranches and 856 143 were captive bred. (Wild harvest refers to hunting or harvest directly from a wild population; ranching refers to crocodiles raised on farms, but collected in the wild either as eggs or hatchlings. Captive bred, or farmed crocodiles, are hatched from eggs originating from breeding stock kept on the farm). Eight of the 23 crocodilian species are currently used in the worldwide industry. The Nile crocodile (*Crocodylus niloticus*) is the species most utilized in Africa. Wild harvest still occurs in Tanzania, while ranching occurs in Botswana, Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Namibia, Tanzania, Uganda, Zambia and Zimbabwe. Captive breeding occurs in Kenya, Madagascar, Namibia, South Africa, Botswana and Zimbabwe. In 1999, Nile crocodile skins accounted for 126 612 out of the total supply of 1 182 469 skins (10.7 %). As such it is the third most important crocodilian from an economic perspective. Table 2.1 demonstrates the increasing importance of farming in the production of Nile crocodile skins.

Table 2.1: Number of *C. niloticus* skins originating from the different sources in 1980 and 1999. (Adapted from MacGregor, 2002)

| Year | Source | | |
|---|---|---|---|
| | Wild | Ranched | Captive bred |
| 1980 | 16 508 | 12 | 0 |
| 1999 | 1 294 | 56 158 | 69 160 |

Experience gained during the early years of crocodile farming in southern Africa has led to much improved husbandry, better survival rates and reduced disease incidence. Hatchling mortality in Zimbabwe reduced from 31.2 % in 1983 to 11.7 % in 1991 (Foggin, 1992a). Nevertheless, certain diseases remain important. Bacterial enteritis and septicaemia are the most common cause of death (Foggin, 1992b). This is a complex syndrome and control depends on optimal husbandry practices. Mycoplasmosis has assumed increasing importance since its initial diagnosis in 1995 (Mohan et al., 1995 and 2001). Several large outbreaks have occurred on South African crocodile farms, with high morbidity and huge financial implications. (Picard, pers comm.). Chlamydiosis recently caused very high mortalities in Indo-Pacific crocodiles (*C. porosus*) (Huchzermeyer et al., 2006).

Veterinary involvement on crocodile farms therefore remains important to limit production loss due to disease. To date, diagnostics have relied primarily on post mortem examinations in the face of a disease outbreak. In veterinary medicine clinical pathology is widely employed as a diagnostic tool in many species. Millan et al. (2000) demonstrated the potential application of reference ranges for *C. porosus* as a basis in disease investigations, and Glassman et al. (1981) demonstrated predictable changes to the heamograms of American alligators infected with *Aeromonas hydrophila*. However, the usefulness of clinical pathology in Nile crocodiles is restricted by the lack of reference ranges for haematological and biochemical values. The limited studies to date have been on farmed Nile crocodiles, which due to their stressed metabolic state, do not necessarily represent true normal values.

The purpose of this study was to establish normal haematological and biochemical values for wild Nile crocodiles, to contribute towards the establishment of reference ranges for *C. niloticus*. Such ranges will make the interpretation of laboratory results easier, making clinical pathology a viable diagnostic tool for Nile crocodiles in both the zoo and the crocodile farm environment. The other application of this baseline data is for ecological monitoring. Crocodiles are terminal predators, and as such are good indicators of the state

of their environment. Consequently the crocodile is an ideal species to study to assess the ecology of a system. As so little is known about wild crocodile physiology at this stage, a certain amount of baseline data is essential for future comparisons.

**Study site**

Botswana's Okavango Delta, the world's biggest RAMSAR site, is a massive wetland within the Kalahari Desert, covering an area of approximately 16 000 $km^2$ in the dry season, and increasing to over 22 000 $km^2$ with the annual flood. The 111 250 $km^2$ active catchment area falls entirely within Angola. Due to the geology of the catchment, the incoming water is low in nutrients and sediment. (Mendelsohn and Obeid, 2004).

The Okavango River flows through Namibia briefly before entering Botswana and forming a broad floodplain, the Panhandle. An estimated 40 % of incoming water leaks into the surrounding swamps by the time the river leaves the Panhandle. The remaining 60 % is distributed down three main channels, which fan out to form the Delta. The Okavango Delta consists of permanent and seasonal swamp, which is inundated during the annual flood.

The northern part of the Delta is characterized by shallow water, flooded grasslands, ox-bow lakes and lagoons mostly interconnected by narrow waterways. Only a few main channels lined by tall reeds (mainly *Phragmites australis*), carry the remainder of the Okavango's water southwards through the Delta. The permanent and seasonal swamp, together form a unique ecosystem, providing high quality habitat for a great many species. As a keystone species, the Nile crocodile helps maintain the fragile balance within this ecosystem. Crocodiles are unevenly distributed throughout the Delta, with the majority of the breeding population occurring in the 120 km long Panhandle.

**Study Animal**

The Nile crocodile, *Crocodylus niloticus*, is the most widespread and abundant of the three crocodile species that occur in Africa. It occurs throughout the continent south of the Sahara, in a variety of wetland habitats, including coastal areas (Taplin and Loveridge, 1988). Historically its distribution in southern Africa extended down the east coast as far as the Kei River.

The Nile crocodile is dark olive to brown, with a light yellow abdomen. Being reptiles, they are ectothermic and regulate their body temperature behaviourally, by moving between sun-exposed sandbanks and the water. Typical adult lengths are around 3.5 m, but

they can grow up to 5 m (Groombridge, 1987). Sexual maturity is reached from 2.9 m total length for males, and 2.2 m for females (Cott, 1961). Nesting occurs in a hole in the ground, where an average of 50 eggs are laid. Nile crocodiles exhibit temperature-dependent sex determination (Leslie, 1997; Hutton, 1987). Hatchlings emerge after an incubation period of approximately 90 days, in early to mid summer, and parental protection occurs (Pooley and Gans, 1976; Pooley, 1977). There is a very high mortality rate in their first year of life due to predation.

## Materials and Methods

### Capture method

Crocodiles were captured in the Panhandle of the Okavango during summer (February 2005). Capture was carried out at night, using a 4.8 m flat bottomed aluminium boat propelled by a 60 hp engine. Crocodiles were located using a 500 000 candle power spot-light which, once shone into the crocodile's eyes, reflected back a red glow due to the presence of a retinal tapetum lucidum. Once spotted, the beam of light was kept focused on the crocodile's eyes, making it possible to approach the animal by boat. Crocodiles estimated to be smaller than 1.2 m total length (TL) were captured by hand. Crocodiles between 1.2 m and 2.3 m were captured using a swivelling noose (Animal Handling Co.) which was placed over the snout and pulled tight in the neck region. Crocodiles were then brought onto the boat, jaws were taped shut and the animals were physically restrained. Animals larger than 2.3 m were captured using a noose attached to a climbing rope, which was secured to the boat. The crocodile was allowed to swim so as to tire it out before it was brought onto the boat.

### Blood collection and biological measurements

Each crocodile was blindfolded and restrained in ventral recumbency. Fifty three animals were randomly selected for blood collection. Blood was collected from the post occipital sinus (Campbell, 1996), on the dorsal midline and just caudal to the base of the head. A 21 G or 23 G needle and a three, five or 10 ml syringe was used, depending on the size of the crocodile, and the blood was transferred directly into a lithium heparin blood tube. Blood smears were made from whole blood using the cover slip method (Jain, 1986).

Following blood collection the crocodile was measured: total length (TL) and snout-to-vent length (SVL) were recorded using a flexible measuring tape (±1 mm), and weighed using a harness around the forelimbs and a Pesola spring balance. Each crocodile was

sexed by cloacal examination of the cliteropenis (Hutton, 1987; Leslie, 1997), and examined for clinical abnormalities including bite wounds, skin lesions, conjunctivitis, and poor condition.

**Haematology**

On return to the field laboratory 1.0 ml of blood was transferred to an Epindorf tube for haematological analysis. The remaining blood was centrifuged using a manual desktop centrifuge, and plasma decanted and frozen for biochemistry. If the volume of the blood sample was small it was allocated for either heamatological or biochemical analysis, but not both. Haematological analysis was performed on 39 samples.

Packed cell volumes (PCV) were determined using a Statspin MP microhaematocrit centrifuge: blood was drawn into a standard microhaematocrit tube and spun for five minutes at 12 000 G.

Total red cell counts (RCC) were performed both manually and automatically using an electronic particle counter. The automated counts were done by a Beckman Coulter Ac*T Series haematology analyzer (Coulter SA). The manual counts were done using Natt and Herrick's solution. A 1:200 dilution was made by drawing blood up to the 0.5 mark on a red blood cell diluting pipette, then filling the pipette to the 101 mark with Natt and Herrick's solution (Campbell, 1996). The diluted blood was then used to charge both counting chambers of an improved Neubauer haemocytometer (Hawksley and Sons, Lancing, UK). After 5 minutes in a damp chamber the red cells were counted in the 4 corner cells and central cell of the central large square of the counting chamber. This was repeated on the second chamber, and the average multiplied by 10 000 to obtain the total red cell count per microliter.

Haemoglobin concentration (Hb) was determined using a Beckman Coulter Ac*T Series haematology analyzer (Coulter SA).

Red blood cell indices were calculated using standard formulas (Jain, 1986):

Mean corpuscular volume: MCV (fl) = PCV/RCC

Mean corpuscular haemoglobin: MCH (pg) = Hb (g/dl) x 10 / RCC

Mean corpuscular haemoglobin concentration: MCHC (g/dl) = Hb (g/dl) / PCV

Total white cell counts (WBC) were obtained indirectly using the Unopette 5877 system (Becton-Dickinson, USA). The Unopette pipette was filled with blood (25µl) and mixed with the phloxine B diluent in the reservoir. From this both counting chambers of an improved Neubauer haemocytometer were charged. After 5 minutes in a damp chamber all the pink staining granulocytes were counted in both chambers.

Blood smears were stained with Diff-Quick stain (American Scientific Products, Illinois, USA), (Campbell, 1995). Differential leukocyte counts were done on the stained smears. The percentage of heterophils and eosinophils was calculated and used to calculate total WBC (Campbell, 1996):

$$\text{Total WBC/µl} = \frac{\text{stained cells counted in chambers} \times 1.1 \times 16 \times 100}{\text{percentage heterophils and eosinophils.}}$$

Thrombocytes were not counted. If included in a percentage differential count it tends to upset the other values.

The prevalence of haemoparasites was determined on the Diff-Quick stained blood smears.

**Biochemistry**

Epindorf tubes containing plasma were stored at -10 °C in a domestic gas freezer for up to one month. On return from the study site 35 samples were submitted to the laboratory[1]. Biochemical analyses were performed on a Next/Vetex Alfa Wassermann Analyser (Alfa Wassermann B.V., Woerden, The Netherlands). Total protein was determined by a modified Wechselbaums biuret method and albumin by the bromocresol green method. Globulin and albumin:globulin ratio were calculated. Creatinine was determined by the picrate method, total calcium by the Arsenazo method, and glucose by the glucose oxidase method. Cholesterol was determined by enzymatic methods. Magnesium was measured by the zylidyl blue method, and uric acid by the uricase method.

Alkaline phosphatase (ALP), alanine aminotranferase (ALT) and aspartate aminotransferase (AST) were determined by optimized versions of the standard IFCC methods.

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[1] Clinical Pathology Laboratory, Onderstepoort Veterinary Academic Hospital.

Sodium, Potassium, Chloride and ionised Calcium were measured using an 865 pH/Blood Gas Analyser (Chiron Diagnostics Limited, Halstead), by means of ion selective electrodes.

**Statistical analysis**

All values were analyzed for significant differences ($p < 0.05$) between sexes and between size classes by one way analysis of variance (ANOVA). The residuals were checked for normality of distribution with normal probability plots. Where data was not normally distributed, significance was tested by a Mann-Whitney test (gender comparison), and by a non-parametric Kruskall-Wallis test (size comparison).

**Results**

**Haematology**

One specimen had a severely elevated WBC (60.90 x $10^3/\mu l$), 5.4 times the mean and was excluded from the following results. This crocodile was not identified as sick on clinical examination, nor did its length to weight ratio (1.40) differ from the mean of its size class (1.44). The remaining 38 specimens used for haematology originated from crocodiles ranging in total length from 557 - 1930 mm, with a mean TL of 879 mm, and a mean SVL of 426 mm.

The haematology results are shown in Table 2.2. The mean packed cell volume was 17.9 %. There was no difference between the mean RCC obtained from the manual and automated counts (0.59 x $10^6/\mu l$). The mean erythrocyte indices were 312.2 fl, 123.2 pg and 39.6 g/dl for MCV, MCH and MCHC respectively. The mean WBC was 11.28 x$10^3/\mu l$ with a range of 3.75 - 26.22 x $10^3/\mu l$. The differential count mean values were heterophils 20.5 %, lymphocytes 62.0 %, monocytes 0.9 %, eosinophils 4.9 %, basophils 5.9 %, and azurophils 5.1 %. The differential count includes azurophils. The exact nature of azurophils in reptiles is uncertain (Waters, 1999) and the existence of azurophils in crocodilians is controversial (Mateo et al., 1983). However, we found a line of cells distinct from monocytes that we could not classify except in their own category.

Table 2.2: Normal haematological values of Okavango Nile crocodiles (n=38)

| Parameter | Mean | SD | Range |
|---|---|---|---|
| Total length (mm) | 879.0 | | 557-1930 |
| Snout-vent length (mm) | 426.0 | | 255-1015 |
| Mass (g) | 2879.2 | | 240-25000 |
| PCV (%) | 17.9 | ± 2.0 | 14-22 |
| RCC ($x10^6/\mu l$) | 0.59 | ± 0.12 | 0.35-1.00 |
| Hb (g/dl) | 7.11 | ± 1.00 | 4.7-9.5 |
| MCV (fl) | 312.2 | ± 60.6 | 200.0-465.1 |
| MCH (pg) | 123.2 | ± 25.1 | 83.8-220.9 |
| MCHC (g/dl) | 39.6 | ± 3.3 | 29.0-47.5 |
| WBC ($x10^3/\mu l$) | 11.28 | ± 4.74 | 3.75-26.22 |
| Heterophils % | 20.5 | ± 8.7 | 4-39 |
| Lymphocytes % | 62.0 | ± 11.3 | 44-85 |
| Monocytes % | 0.9 | ± 1.8 | 0-10 |
| Eosinophils% | 4.9 | ± 4.8 | 0-17 |
| Basophils % | 5.9 | ± 4.5 | 0-16 |
| Azurophils % | 5.1 | ± 4.5 | 0-21 |
| Heterophils ($x10^3/\mu l$) | 2.09 | ± 0.75 | 0.45-3.66 |
| Lymphocytes ($x10^3/\mu l$) | 7.20 | ± 3.80 | 1.65-17.83 |
| Monocytes ($x10^3/\mu l$) | 0.09 | ± 0.18 | 0.0-0.79 |
| Eosinophils ($x10^3/\mu l$) | 0.53 | ± 0.56 | 0.0-2.14 |
| Basophils ($x10^3/\mu l$) | 0.69 | ± 0.66 | 0.0-2.90 |
| Azurophils ($x10^3/\mu l$) | 0.60 | ± 0.74 | 0.0-3.93 |

Table 2.3 compares the haematological values of the males and females. The sex ratio was unequal, with 27 males and 9 females. There was a significant difference ($p < 0.05$) in the mean RCC between the sexes. Males had 0.57 x $10^6$ erythrocytes/µl, and females 0.66 x $10^6$/µl. Males had a Hb of 6.90 g/dl, significantly less than females at 7.77 g/dl. The eosinophil percentage and count also differed significantly. Males had 3.8 % and 0.42 x $10^3$ eosinophils/µl, less than half that of females at 9.1 % and 0.97 x $10^3$ eosinophils/µl. Other parameters did not differ significantly.

Table 2.3: Haematology: comparison of male and female Okavango Nile crocodiles. (Mean values in bold differed significantly between males and females.)

| Parameter | Males (n=27) | | | Females (n=9) | | |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Range | Mean | ±SD | Range |
| PCV (%) | 17.7 | ± 1.9 | 14-22 | 19.0 | ± 2.1 | 16-22 |
| RCC ($x10^6$/µl) | **0.57** | ± 0.10 | 0.39-0.74 | **0.66** | ± 0.15 | 0.43-0.72 |
| Hb (g/dl) | 6.90 | ± 0.85 | 4.7-8.2 | 7.77 | ± 1.23 | 6.0-9.5 |
| MCV (fl) | 319.8 | ± 57.9 | 216.2-461.5 | 298.4 | ± 72.4 | 200.0-465.1 |
| MCH (pg) | 124.3 | ± 20.4 | 83.8-182.1 | 122.6 | ± 39.0 | 89.0-220.9 |
| MCHC (g/dl) | 39.1 | ± 3.3 | 29.0-45.0 | 40.8 | ± 3.4 | 37.2-47.5 |
| WBC ($x10^3$/µl) | 11.31 | ± 5.02 | 6.13-26.22 | 10.95 | ± 3.63 | 3.75-15.53 |
| Heterophils % | 20.6 | ± 9.1 | 4-39 | 19.4 | ± 7.0 | 13-35 |
| Lymphocytes % | 63.0 | ± 11.3 | 39-85 | 58.2 | ± 11.7 | 45-72 |
| Monocytes % | 1.0 | ± 2.1 | 0-10 | 0.7 | ± 0.9 | 0-2 |
| Eosinophils% | **3.8** | ± 3.9 | 0-17 | **9.1** | ± 5.4 | 2-16 |
| Basophils % | 5.4 | ± 4.0 | 0-15 | 7.7 | ± 6.1 | 0-16 |
| Azurophils % | 5.3 | ± 5.0 | 0-21 | 4.6 | ± 2.9 | 0-8 |
| Heterophils ($x10^3$/µl) | 2.10 | ± 0.97 | 0.45-3.66 | 1.96 | ± 0.54 | 1.33-2.92 |
| Lymphocytes ($x10^3$/µl) | 7.31 | ± 4.30 | 3.3-17.83 | 6.59 | ± 2.82 | 1.65-10.02 |
| Monocytes ($x10^3$/µl) | 0.10 | ± 0.20 | 0.0-0.79 | 0.07 | ± 0.08 | 0.0-0.19 |
| Eosinophils ($x10^3$/µl) | **0.42** | ± 0.46 | 0.0-2.14 | **0.97** | ± 0.66 | 0.0-1.87 |
| Basophils ($x10^3$/µl) | 0.64 | ± 0.63 | 0.0-2.90 | 0.86 | ± 0.79 | 0.0-2.49 |
| Azurophils ($x10^3$/µl) | 0.63 | ± 0.82 | 0.0-3.93 | 0.47 | ± 0.32 | 0.0-0.99 |

Table 2.4 compares the haematological values of the different size classes. The size classes were determined by SVL: yearlings 170-389 mm, juveniles 390-663 mm and subadults 664-1158 mm. The sample size in each size class was not equal, there being 22 yearlings, 11 juveniles and only 5 subadults. There were a number of significant differences ($p < 0.05$). The mean PCV of the yearlings was lower than the subadults (17.3 % compared to 20.2 %). Hb was lower in yearlings (6.73 g/dl) and juveniles (7.16 g/dl) than in subadults (8.62 g/dl). Eosinophil percentage of both yearlings (2.8 %) and juveniles (5.9 %) was lower than that of subadults (12 %) and the eosinophil count differed significantly between all three size classes (yearlings 0.27 x $10^3$ /µl, juveniles 0.68 x $10^3$ /µl, subadults 1.35 x $10^3$ /µl). Basophil percentage of yearlings (4.4 %) and juveniles (6.1 %) differed from subadults (12.0 %) and the basophil count differed between yearlings and subadults.

Haemogregarine parasites were present in 21 out of 38 specimens (55.3%). There was no significant difference between the mean packed cell volume of the infected group at 18.2 %, compared to 17.6 % in the uninfected group. The mean SVL of the infected crocodiles was 463 mm compared to 382 mm for the uninfected crocodiles.

Table 2.4: Haematology: comparison of different size classes.

(SVL: yearlings: 170-389 mm, juveniles: 390-663 mm, subadults: 664-1158 mm)

| Parameter | Yearlings (n=22) | | | Juveniles(n=11) | | | Subadults (n=5) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range | Mean | SD | Range |
| PCV (%) | **17.3** | 1.8 | 14-21 | 18.2 | 1.5 | 16-22 | **20.2** | 2.0 | 17-22 |
| RCC ($x10^6/\mu l$) | 0.58 | 0.11 | 0.35-0.74 | 0.58 | 0.06 | 0.47-0.66 | 0.70 | 0.21 | 0.61-1.00 |
| Hb (g/dl) | **6.73** | 0.83 | 4.7-8.2 | **7.16** | 0.67 | 6.0-8.6 | **8.62** | 0.90 | 7.1-9.5 |
| MCV (fl) | 311.0 | 62.3 | 216.2-461.5 | 315.3 | 41.0 | 242.4-383.0 | 311.0 | 96.4 | 200.0-465.1 |
| MCH (pg) | 120.1 | 21.5 | 83.8-182.1 | 124.2 | 16.2 | 90.9-149.0 | 134.2 | 50.4 | 89.0-220.9 |
| MCHC (g/dl) | 38.9 | 3.6 | 29.0-45.0 | 39.4 | 1.6 | 37.2-42.2 | 42.8 | 3.2 | 39.5-47.5 |
| WBC ($x10^3/\mu l$) | 10.12 | 3.63 | 6.13-19.61 | 13.53 | 6.61 | 3.75-26.22 | 11.45 | 2.83 | 7.84-15.53 |
| Heterophils % | 22.0 | 9.0 | 4-39 | 19.4 | 9.3 | 8-35 | 16.8 | 4.8 | 13-25 |
| Lymphocytes % | 64.1 | 10.8 | 49-85 | 61.9 | 12.6 | 39-83 | 53.0 | 6.4 | 45-58 |
| Monocytes % | 1.2 | 2.2 | 0-10 | 0.4 | 0.9 | 0-3 | 0.6 | 0.9 | 0-2 |
| Eosinophils% | **2.8** | 3.1 | 0-11 | **5.9** | 4.9 | 1-17 | **12.0** | 3.7 | 7-16 |
| Basophils % | **4.4** | 3.3 | 0-13 | **6.1** | 4.6 | 1-15 | **12.0** | 4.2 | 7-16 |
| Azurophils % | 5.0 | 5.0 | 0-21 | 5.1 | 4.6 | 0-15 | 5.2 | 2.2 | 3-8 |
| Heterophils ($x10^3/\mu l$) | 2.11 | 0.85 | 0.45-3.66 | 2.14 | 0.67 | 1.31-3.43 | 1.87 | 0.47 | 1.33-2.59 |
| Lymphocytes ($x10^3/\mu l$) | 6.64 | 3.08 | 3.30-14.71 | 8.79 | 5.25 | 1.65-17.83 | 6.19 | 2.11 | 3.53-9.01 |
| Monocytes ($x10^3/\mu l$) | 0.10 | 0.17 | 0.0-0.70 | 0.09 | 0.24 | 0.0-0.79 | 0.06 | 0.08 | 0.0-0.16 |
| Eosinophils ($x10^3/\mu l$) | **0.27** | 0.34 | 0.0-1.41 | **0.68** | 0.57 | 0.20-2.14 | **1.35** | 0.42 | 0.72-1.87 |
| Basophils ($x10^3/\mu l$) | **0.45** | 0.36 | 0.0-1.24 | 0.85 | 0.86 | 0.15-2.9 | **1.38** | 0.68 | 0.87-2.49 |
| Azurophils ($x10^3/\mu l$) | 0.51 | 0.52 | 0.0-1.92 | 0.81 | 1.18 | 0.0-3.93 | 0.56 | 0.20 | 0.37-0.89 |

**Biochemistry**

The 35 specimens that underwent biochemical analysis originated from crocodiles which ranged from 593-1930 mm TL, with a mean of 916 mm, and a mean SVL of 449 mm.

The results of the biochemistry are shown in Table 2.5. Mean total protein, albumin and globulin were 41.2, 14.7, and 26.5 g/l respectively. ALT, ALP and AST were 43.9, 21.1 and 66.5 U/l respectively. Mean Glucose was 3.8 mmol/l, sodium was 147.9 mmol/l and potassium was 4.88 mmol/l. Mean ionized calcium and total calcium were 1.35 mmol/l and 2.73 mmol/l respectively. Mean magnesium was 1.15 mmol/l, cholesterol 5.49 mmol/l, and creatinine 34.0 µmol/l. Mean chloride was 120.3 mmol/l, and mean uric acid 0.12 mmol/l.

Table 2.5: Normal biochemical values of Okavango Nile crocodiles. (n=35)

| Parameter | Mean | | SD | Range |
|---|---|---|---|---|
| Total length (mm) | 916.0 | | | 593-1930 |
| Mass (g) | 3208.0 | | | 305-25000 |
| Total protein (g/l) | 41.2 | | | 28.9-57.1 |
| Alb (g/l) | 14.7 | ± | 1.8 | 11.1-19.4 |
| Glob (g/l) | 26.5 | ± | 6.8 | 16.5-42.6 |
| A:G ratio | 0.58 | ± | 0.12 | 0.34-0.79 |
| ALT (U/l) | 43.9 | ± | 13.1 | 15-63 |
| ALP (U/l) | 21.1 | ± | 13.7 | 3-72 |
| AST (U/l) | 66.5 | ± | 56.4 | 14-211 |
| Gluc (mmol/l) | 3.8 | ± | 0.5 | 1.8-4.8 |
| Na (mmol/l) | 147.9 | ± | 8.3 | 122-164 |
| K (mmol/l) | 4.88 | ± | 1.03 | 3.30-7.65 |
| $Ca^{Total}$ (mmol/l) | 2.73 | ± | 0.19 | 2.34-3.15 |
| $Ca^{2+}$ (mmol/l) | 1.35 | ± | 0.12 | 1.08-1.61 |
| Mg (mmol/l) | 1.15 | ± | 0.26 | 0.65-1.72 |
| Chol (mmol/l) | 5.49 | ± | 2.08 | 0.0-9.86 |
| Creat (µmol/l) | 34.0 | ± | 10.2 | 17-56 |
| Cl (mmol/l) | 120.3 | ± | 9.6 | 97-135 |
| UA (mmol/l) | 0.12 | ± | 0.05 | 0.04-0.30 |

Table 2.6 is a comparison between males and females. The sex distribution was not equal, with 27 males and eight females. Mean total protein of males was significantly lower than that of females (39.82 g/l compared to 45.83 g/l), and the albumin:globulin ratio in males was higher than females (0.61 compared to 0.47). Potassium was significantly lower in males (4.65 mmol/l) than females (5.68 mmol/l). The other parameters showed no significant differences between the sexes.

Table 2.6: Biochemistry: comparison of male and female Okavango Nile crocodiles. (Mean values in bold differed significantly between males and females)

| Parameter | Males (n= 27) | | | Females (n=8) | | |
|---|---|---|---|---|---|---|
| | Mean | ±SD | Range | Mean | ±SD | Range |
| Tot protein (g/l) | **39.82** | ± 6.18 | 28.9-52.8 | **45.83** | ± 10.37 | 33.8-57.1 |
| Alb (g/l) | 14.84 | ± 1.77 | 11.9-19.4 | 14.09 | ± 1.70 | 11.1-15.1 |
| Glob (g/l) | 24.99 | ± 5.20 | 16.5-36.6 | 31.78 | ± 9.20 | 22.1-42.6 |
| A:G ratio | **0.61** | ± 0.11 | 0.43-0.79 | **0.47** | ± 0.11 | 0.34-0.61 |
| ALT (U/l) | 45.2 | ± 12.14 | 23-69 | 39.3 | ± 16.14 | 15-63 |
| ALP (U/l) | 22.7 | ± 14.34 | 3-72 | 16.0 | ± 10.42 | 3-32 |
| AST (U/l) | 57.4 | ± 50.35 | 14-189 | 97.3 | ± 67.95 | 14-211 |
| Gluc (mmol/l) | 3.87 | ± 0.60 | 3.4-4.8 | 3.71 | ± 0.29 | 3.3-4.1 |
| Na (mmol/l) | 146.79 | ± 7.28 | 143-158 | 151.80 | ± 10.80 | 129-164 |
| K (mmol/l) | **4.65** | ± 0.82 | 3.30-6.55 | **5.68** | ± 1.31 | 4.21-7.65 |
| $Ca^{Total}$ (mmol/l) | 2.72 | ± 0.17 | 2.38-3.05 | 2.78 | ± 0.26 | 2.34-3.15 |
| $Ca^{2+}$ (mmol/l) | 1.37 | ± 0.11 | 1.15-1.61 | 1.29 | ± 0.15 | 1.08-1.45 |
| Mg (mmol/l) | 1.17 | ± 0.25 | 0.65-1.72 | 1.09 | ± 0.27 | 0.73-1.55 |
| Chol (mmol/l) | 5.67 | ± 2.03 | 0-8.69 | 4.88 | ± 2.24 | 2.88-9.86 |
| Creat (µmol/l) | 34.00 | ± 10.98 | 17-56 | 34.13 | ± 7.32 | 21-46 |
| Cl (mmol/l) | 120.70 | ± 9.30 | 97-135 | 119.14 | ± 11.39 | 107-133 |
| UA (mmol/l) | 0.13 | ± 0.05 | 0.04-0.18 | 0.11 | ± 0.04 | 0.05-0.15 |

Table 2.7 compares the biochemical values of the different size classes. The distribution between the size classes was not equal, with 17 yearlings, 13 juveniles and only five subadults.

Mean total protein of yearlings (37.73 g/l) and juveniles (41.41 g/l) were both significantly lower than subadults (52.42 g/l). Mean globulin of yearlings (23.34 g/l) and juveniles (26.62 g/dl) were both lower than subadults (37.24 g/dl). A:G ratio was significantly higher in yearlings and juveniles compared to subadults. AST of yearlings was significantly lower than that of subadults (36.59 U/l compared to 135.0 U/l). Glucose was higher in yearlings (4.12 mmol/l) than in juveniles (3.54 mmol/l). Sodium in yearlings (146.17 mmol/l) was significantly lower than in subadults (156.88 mmol/l). Potassium was significantly lower in both yearlings (4.44 mmol/l) and juveniles (4.92 mmol/l) than in subadults (6.30 mmol/l).

Table 2.7: Biochemistry: comparison of different size classes.

(SVL: Yearlings: 170-389 mm, juvenile: 390-663 mm, subadults: 664-1158 mm)

| Parameter | Yearlings (n=17) | | | Juveniles(n=13) | | | Subadults (n=5) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range | Mean | SD | Range |
| Tot protein (g/l) | **37.73** | 6.05 | 28.9-52.8 | **41.41** | 5.63 | 33.4-52.5 | **52.42** | 6.51 | 41.4-57.1 |
| Alb (g/l) | 14.42 | 1.75 | 11.9-17.4 | 14.79 | 2.09 | 11.1-19.4 | 15.18 | 0.64 | 14.3-15.9 |
| Glob (g/l) | **23.34** | 4.73 | 16.5-36.4 | **26.62** | 4.84 | 19.5-36.6 | **37.24** | 6.95 | 25.7-42.6 |
| A:G ratio | **0.63** | 0.09 | 0.45-0.79 | **0.57** | 0.12 | 0.43-0.77 | **0.42** | 0.11 | 0.34-0.61 |
| ALT (U/l) | 45.35 | 10.62 | 26-63 | 45.54 | 14.20 | 23-69 | 34.40 | 16.80 | 15-54 |
| ALP (U/l) | 20.82 | 11.59 | 3-54 | 22.31 | 17.56 | 5-72 | 19.20 | 11.08 | 3-32 |
| AST (U/l) | **36.59** | 33.67 | 14-149 | 79.23 | 55.87 | 14-189 | **135.00** | 54.05 | 59-211 |
| Gluc (mmol/l) | **4.12** | 0.37 | 3.4-4.8 | **3.54** | 0.64 | 1.8-4.5 | 3.60 | 0.29 | 3.3-4.0 |
| Na (mmol/l) | **146.17** | 4.47 | 137.9-157.0 | 146.81 | 10.79 | 122.0-158.0 | **156.88** | 6.39 | 148.0-164.0 |
| K (mmol/l) | **4.44** | 0.73 | 3.30-5.96 | **4.92** | 0.80 | 3.89-6.55 | **6.30** | 1.28 | 4.21-7.65 |
| $Ca^{Total}$ (mmol/l) | 2.69 | 0.16 | 2.38-2.90 | 2.74 | 0.20 | 2.34-3.05 | 2.86 | 0.24 | 2.63-3.15 |
| $Ca^{2+}$ (mmol/l) | 1.33 | 0.09 | 1.14-1.49 | 1.39 | 0.14 | 1.15-1.61 | 1.31 | 0.16 | 1.08-1.44 |
| Mg (mmol/l) | 1.10 | 0.26 | 0.65-1.72 | 1.19 | 0.28 | 0.73-1.57 | 1.24 | 0.19 | 1.03-1.55 |
| Chol (mmol/l) | 6.36 | 2.40 | 0.00-9.86 | 4.74 | 1.42 | 2.88-7.80 | 4.47 | 1.07 | 2.91-5.49 |
| Creat (µmol/l) | 31.82 | 11.16 | 17-50 | 36.77 | 10.30 | 19-56 | 34.40 | 3.91 | 29-40 |
| Cl (mmol/l) | 122.71 | 6.19 | 112-135 | 118.17 | 12.19 | 97-133 | 118.50 | 11.68 | 107-130 |
| UA (mmol/l) | 0.13 | 0.04 | 0.05-0.20 | 0.12 | 0.07 | 0.04-0.30 | 0.09 | 0.01 | 0.08-0.10 |

Table 2.8 is a comparison of the results to this study to those previously reported from farmed Nile crocodiles. The packed cell volume of the Okavango population was substantially lower than that reported from farmed Nile crocodiles

Table 2.8: Haematology: comparison of wild and farmed Nile crocodiles.

| | This study | | Makinde et al 1991 | | Foggin 1987[1] | | Thurman 1990 |
|---|---|---|---|---|---|---|---|
| Parameter | Mean | Range | Mean | Range (n=44) | Mean | Range | Mean (n=5) |
| PCV (%) | 17.9 | 14-22 | 27.2 | 24-31 | 22 | 13-27 | |
| RCC (x10to6/µl) | 0.59 | 0.35-1.00 | 0.92 | 0.6-1.31 | | | |
| Hb (g/dl) | 7.11 | 4.7-9.5 | 8.7 | 7.8-9.5 | 7.4 | 6.4-8.7 | |
| MCV (fl) | 312.2 | 200.0-465.1 | 306.7 | 206.1-440.7 | | | |
| MCH (pg) | 123.2 | 83.8-220.9 | 97.9 | 65.3-153.3 | | | |
| MCHC (g/dl) | 39.6 | 29.0-47.5 | 31.9 | 29.0-38.3 | | | |
| WBC (x10³/µl) | 11.28 | 3.75-26.22 | 6.4 | 4.0-11.5 | | | |
| Heterophils % | 20.5 | 4-39 | 13.4 | 6-20 | | | 50 |
| Lymphocytes % | 62.0 | 44-85 | 82.2 | 73-95 | | | 21 |
| Monocytes % | 0.9 | 0-10 | 2.5 | 1-7 | | | 5 |
| Eosinophils% | 4.9 | 0-17 | 4.4 | 2-8 | | | 2 |
| Basophils % | 5.9 | 0-16 | | | | | 22 |
| Azurophils % | 5.1 | 0-21 | | | | | |
| Heterophils (x10³/µl) | 2.09 | 0.45-3.66 | | | | | |
| Lymphocytes (x10³/µl) | 7.20 | 1.65-17.83 | | | | | |
| Monocytes (x10³/µl) | 0.09 | 0-0.79 | | | | | |
| Eosinophils (x10³/µl) | 0.53 | 0-2.14 | | | | | |
| Basophils (x10³/µl) | 0.69 | 0-2.90 | | | | | |
| Azurophils (x10³/µl) | 0.60 | 0-3.93 | | | | | |

[1] n was not provided.

Table 2.9 shows a comparison of haematological values from various other crocodilian species. In comparison to other crocodilian species, the values reported in this study are generally within the ranges recorded for other crocodilians

Table 2.9: Haematological values from various crocodilian species.

| Parameter | *C. niloticus*[1] mean | *C. niloticus*[1] range | *C. rhom*[2] | *C. poro*[3] | *C. poro*[4] | *C. john*[5] | *A. mississip*[6] | *C. palus*[8] | *Ca. lati*[10] | *Ca. croc*[11] |
|---|---|---|---|---|---|---|---|---|---|---|
| PCV (%) | 17.9 | 14-22 | 23.6-25.8 | 20-22 | 17-41 | 18-21 | 18.6 | 24.9 | 22 | 27 |
| RCC (x10$^6$/µl) | 0.59 | 0.35-1.00 | | 0.86-0.98 | 0.6-1.3 | 0.71-0.93 | 0.4 | 0.69 | 0.56 | 0.69 |
| Hb (g/dl) | 7.11 | 4.7-9.5 | 8.1-8.9 | 6.2-7.7 | 4.7-12.2 | 5.7-7.5 | 7.2 | 8.3 | 9.4 | 12 |
| MCV (fl) | 312.2 | 200.0-465.1 | | | 240-311 | | 516.0 | 362.4 | | |
| MCH (pg) | 123.2 | 83.8-220.9 | | | 72-92 | | 185.0 | 120.7 | | |
| MCHC (g/dl) | 39.6 | 29.0-47.5 | | | 26.1-31.9 | | 36.1 | 33.4 | | |
| WBC (x10$^3$/µl) | 11.28 | 3.75-26.22 | | 39.6-44.2 | 6.4-25.7 | 26.4-48.8 | 5.3 | 8.71 | 22.7 | 16.4 |
| WBC (x10$^3$/µl) | | | | 5.33[9] | | | 6.4[7] | | | |
| Heterophils % | 20.5 | 4-39 | | | | | 37.4, 54.7[7] | | 3 | 5 |
| Lymphocytes % | 62.0 | 44-85 | | | | | 50.6, 23.9[7] | | 65 | 60 |
| Monocytes % | 0.9 | 0-10 | | | | | 3.0, 0.7[7] | | 5 | 5 |
| Eosinophils% | 4.9 | 0-17 | | | | | 5.5, 10.4[7] | | 19 | 21 |
| Basophils % | 5.9 | 0-16 | | | | | 3.5, 12.7[7] | | 1 | 0 |
| Azurophils % | 5.1 | 0-21 | | | | | | | 8 | 9 |
| Heterophils (x10$^3$/µl) | 2.09 | 0.45-3.66 | | 3.08[9] | 0.8-7.4 | | | 5.6 | | |
| Lymphocytes (x10$^3$/µl) | 7.20 | 1.65-17.83 | | 1.69[9] | 4.5-21.6 | | | 2.48 | | |
| Monocytes (x10$^3$/µl) | 0.09 | 0.0-0.79 | | 0.05[9] | 0.0-1.2 | | | 0.09 | | |
| Eosinophils (x10$^3$/µl) | 0.53 | 0.0-2.14 | | 0.35[9] | 0.0-0.7 | | | 0.53 | | |
| Basophils (x10$^3$/µl) | 0.69 | 0.0-2.90 | | 0.15[9] | 0.0-0.4 | | | 0.01 | | |
| Azurophils (x10$^3$/µl) | 0.60 | 0.0-3.93 | | | | | | | | |

[1] *C. niloticus*: This study (means); [2] *C. rhombifer*: Carmena-Suero et al., 1979, n=19, subadults (range of means);

[3] *C. porosus*: Canfield, 1985, n=4, subadults (ranges); [4] *C. porosus*: Millan et al., 1997, n=29, yearlings (ranges);

[5] *C. johnstoni*: Canfield, 1985, n=4, subadults (ranges); [6] *A. mississippiensis*: Glassman et al., 1981, n=45 (means);

[7] *A. mississippiensis*: Mateo et al., 1984, n=35 (means); [8] *C. palustris*: Stacey and Whitaker, 2000, n=24, juveniles (means);

[9] *C. porosus*: Turton et al., 1997, n=140, hatchlings (means); [10] *Caiman latirostris*: Troiano et al., 1996 (means);

[11] *Caiman crocodilus*: Troiano et al., 1996 (means).

Table 2.10 shows the reported biochemical values for Nile crocodiles from various other localities within southern Africa, and Table 2.11 shows reported biochemical values for other species of crocodilians.

Table 2.10: Comparison of biochemical values from Nile crocodiles in various locations.

| Parameter | Okavango[1] mean | Okavango[1] range | KNP[2] | St. Lucia[3] | SA captive[4] | SA farms[5] | Zim farms[6] |
|---|---|---|---|---|---|---|---|
| Tot prot. (g/l) | 41.2 | 28.9-57.1 | | | 50 ± 3 | 50.2 | 53.0 |
| Alb (g/l) | 14.7 | 11.1-19.4 | 9.8-16.38 | | | | 19.0 |
| Glob (g/l) | 26.5 | 16.5-42.6 | 30.7-47.35 | | | | 31.0 |
| A:G ratio | 0.58 | 0.34-0.79 | | | | | |
| ALT (U/l) | 43.9 | 15-63 | | | | | 13.1 |
| ALP (U/l) | 21.1 | 3.-72 | | | | 64.2 | |
| AST (U/l) | 66.5 | 14-211 | | | | | 16.6 |
| Gluc (mmol/l) | 3.8 | 1.8-4.8 | 3.2-11.45 | | 5.9 ± 0.9 | 5.9 | 4.57 |
| Na (mmol/l) | 147.9 | 122-164 | 141.5-154.5 | 141.8-160.7 | 154.0 ± 1.00 | 153.8 | |
| K (mmol/l) | 4.88 | 3.30-7.65 | 2.53-5.35 | 3.2-5.8 | 3.8 ± 0.50 | 3.8 | |
| Ca (mmol/l) | 2.73 | 2.34-3.15 | 2.6-3.98 | | 2.97 ± 0.09 | 2.97 | 2.63 |
| Mg (mmol/l) | 1.15 | 0.65-1.72 | 1.51-2.24 | | 0.9 ± 0.10 | 0.52 | |
| Chol (mmol/l) | 5.49 | 0-9.86 | | | 35.8 ± 5.4 | | |
| Creat (µmol/l) | 34.0 | 17-56 | 36.5-97.0 | | | | |
| Cl (mmol/l) | 120.3 | 97-135 | 88.5-120.5 | 86.0-118.6 | | | |
| UA (mmol/l) | 0.12 | 0.04-0.30 | | | | | 0.24 |

[1] This study, n=35

[2] Swannepoel et al., 2000, n=14 (range of means)

[3] Leslie, 1997, n=80 (range of means)

[4] Watson, 1990, n=5 (means ± SD)

[5] Thurman, 1990, n=5 (means)

[6] Foggin, 1987, n = not provided (means).

Table 2.11: Biochemical values from various crocodilian species.

| Parameter | *C. niloticus*[1] | | *C. porosus*[2] | *C. palustris*[3] | *A. missisip.*[4] | *T. schlegelii*[5] | *Ca. latirostris*[6] | *C. moreletii*[7] |
|---|---|---|---|---|---|---|---|---|
| | mean | range | | | | | | |
| Total protein (g/l) | 41.2 | 28.9-57.1 | 41-70 | 31.2 | 37.8 | 37 | 50.1 | 93.7 |
| Alb (g/l) | 14.7 | 11.1-19.4 | 14-23 | 11.4 | 10.3 | | 24.2 | |
| Glob (g/l) | 26.5 | 16.5-42.6 | 27-50 | 19.8 | 27.5 | | 31.0 | |
| A:G ratio | 0.58 | 0.34-0.79 | 0.3-0.7 | | | | | |
| ALT (U/l) | 43.9 | 15-63 | 11-51 | 52.63 | 34.35 | | | |
| ALP (U/l) | 21.1 | 3.-72 | 31-180 | 52.75 | 22.82 | 17.8 | | |
| AST (U/l) | 66.5 | 14-211 | 23-157 | 52.13 | 141.42 | | | |
| Gluc (mmol/l) | 3.8 | 1.8-4.8 | 4.5-12.1 | 3.63 | 3.6 | 4.2 | 5.72 | 2.97 |
| Na (mmol/l) | 147.9 | 122-164 | 143-161 | 143.17 | 140.99 | 155.9 | | |
| K (mmol/l) | 4.88 | 3.30-7.65 | 3.8-7.2 | 8.0 | 5.93 | 4.4 | | |
| Ca (mmol/l) | 2.73 | 2.34-3.15 | 2.41-3.45 | 3.18 | 2.31 | 2.55 | 2.53 | 2.63 |
| Mg (mmol/l) | 1.15 | 0.65-1.72 | 0.8-1.4 | | | | | |
| Chol (mmol/l) | 5.49 | 0-9.86 | 1.1-7.2 | 6.4 | 1.71 | 2.86 | 6.0[8] | |
| Creat (µmol/l) | 34.0 | 17-56 | 20-51 | 35.4 | 13.26 | 18.6 | 31.8 | 93.7 |
| Cl (mmol/l) | 120.3 | 97-135 | 88-127 | 119.71 | 112.1 | 120.0 | | |
| UA (mmol/l) | 0.12 | 0.04-0.30 | 0.17-0.99 | 0.35 | 0.25 | 0.19 | | 0.49 |

[1] This study; [2] Millan et al., 1997, n=120 (ranges); [3] Stacey and Whitaker, 2000, juveniles, n=24 (means);

[4] *A. mississipiensis*: Barnett et al., 1998, wild alligators, n=24 (means); [5] Siruntawineti and Ratanakorn, 1994 (means);

[6] Troiano and Althaus, 1993 (means); [7] Sigler, 1991 (means); [8] Tourn et al., 1994. (mean).

## Discussion

### Haematology

The effect of gender and size, on haematological and biochemical values, has been studied in *C. palustris* (Stacey and Whitaker, 2000). There is no clear similarity between the parameters that differed in *C. palustris* compared to those that differed in *C. niloticus*. However, the environmental conditions in the two studies were not comparable: the *C. palustris* were captive specimens.

In *C. palustris* eosinophils differed between size classes, but subadults had lower counts than the juveniles and adults. In the Nile crocodile we found an increasing eosinophil count with increasing size. Causes of an increased eosinophil count in reptiles include parasitic infection and non-specific inflammation (Campbell, 1996; Waters, 1999).

The comparisons between genders and between size classes were done for interest, but were not the original intention of this study. The number of males and females were unequal, as were the number of crocodiles in each size class. The distribution of sexes between the size classes was also unequal. This made it impossible to eliminate the effect of gender when comparing size classes, and impossible to eliminate the effect of size when comparing genders. A future study will require a larger sample size in order to evaluate differences between genders and size classes more accurately.

Comparison of our results with ranges reported previously from Nile crocodiles is difficult because of the limited data available. Likely causes of a low packed cell volume (PCV) include erythrolysis due to haemoparasites, or poor nutritional plane (Wilkinson, 2004; Waters, 1999), or poor collection technique. Over half of our specimens were infected with *Hepatozoon pettiti*. However, there was no significant difference in PCV between the infected and uninfected group. The nutritional plane of wild Okavango crocodiles is undoubtedly lower than that of farmed crocodiles, which frequently become obese. In a comparative study involving wild and farmed American alligators (*A. mississippiensis*), the wild alligators were relatively anaemic, having a lower PCV of 24.44 %, compared to 27.29 %, and a RCC of 0.42 x $10^6$ /µl compared to 0.47 x $10^6$ /µl in the farmed alligators (Barnett et al., 1999). The wild alligators were transferred into captivity. Under captive conditions the alligators became less anaemic.

The WBC of Nile crocodiles in Botswana was higher than that reported by Makinde and Alemu (1991). Causes of increased WBC include inflammatory response to microbial

infection, parasites, and non-specific inflammation (Campbell, 1996; Waters, 1999). In some species of reptiles there is seasonal fluctuation (increase in summer) (Wilkinson, 2004).

Watson (1990) reported a very low mean WBC of 1.5 ± 0.4 x$10^3$ /µl from 5 captive Nile crocodiles. Leukopaenia may result from chronic stress.

In comparison to other crocodilian species, the haematological values reported in this study are generally within the ranges recorded for other crocodilians, although the mean PCV obtained in this study is lower than those reported for other species. The total leukocyte count varies widely between the species.

**Biochemistry**

Effect of gender and size on biochemical parameters again seems variable when compared to *C. palustris* (Stacey and Whitaker, 2000). Adult *C. palustris* had significantly lower AST than the smaller sizes. In contrast, we found AST of subadults was higher than that of yearlings. Both species showed increasing blood glucose with increasing size. Subadult *C. palustris* had lower potassium concentrations than adults and juveniles, while *C. niloticus* showed an increasing potassium concentration with increasing size.

Previously reported biochemical values for Nile crocodiles are shown in Table 2.10. Total protein determined in this study is lower than that reported in farmed Nile crocodiles, which may be caused by a lower nutritional plane, or parasitism (Campbell, 1996). Barnett et al. (1998 and 1999) also found a significantly lower total protein in wild alligators compared to farmed alligators. Mean globulin concentration of the Okavango crocodiles was lower than that in Nile crocodiles in the Kruger National Park, while albumin levels were similar (Swanepoel et al., 2000). This may reflect greater antigenic stimulation of the Kruger specimens (Campbell, 1996).

Blood glucose is lower than that reported in both the farmed and other wild Nile crocodiles. Blood for glucose determination was not collected in fluoride tubes. This may result in lower readings. However, both total protein and glucose were within the ranges reported for certain other species (Table 2.11). Watson (1990) reported a very high mean cholesterol level (35.8 ± 5.4 mmol/l) from five captive Nile crocodiles, but the value determined in this study was similar to that reported in other species. Cholesterol levels

can be influenced by the timing of blood collection relative to a meal. This is usually an unknown factor when collecting from wild crocodiles.

There is considerable variation of both haematological and biochemical values between the different crocodilian species. Clearly a species specific reference range is essential in order to interpret clinical pathology results meaningfully.

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# CHAPTER 3

## Normal Intestinal Flora of Wild Nile Crocodiles (*Crocodylus niloticus*), and the Development and Potential use of a Probiotic in Farmed Crocodiles.

### Introduction

Due to the difficulty in obtaining biological specimens from wild crocodilians, very little is known about their normal intestinal flora. Huchzermeyer et al. (2000) reported on the GIT flora isolated from wild-caught African dwarf crocodiles (*Osteolaemus tetraspis*). Salmonellae isolated from wild Nile crocodiles from Lake Kariba (Madsen et al., 1998), and from wild American alligators (Scott and Foster, 1997) have also been documented. Other studies have dealt with captive crocodilians. Misra et al. (1993) reported the normal intestinal flora of captive gharials (*Gavialis gangeticus*). Prevalence of salmonellae has been studied in healthy captive crocodilians (Obwolo and Zwart, 1993, Manolis et al., 1991).

Farmed crocodile hatchlings often fail to develop a normal mixed intestinal flora (Huchzermeyer, 2003). In other species, the rapid establishment of bacterial communities of normal flora in the gastrointestinal tract (GIT) is thought to be essential for GIT homeostasis and the prevention of colonization by pathogenic bacteria (Dogi and Perdigon, 2006; Nava et al., 2005). A deficient GIT flora is likely to be one of the factors predisposing farmed crocodiles to enteritis. Enteritis, and associated septicaemia, is one of the main causes of mortality in farmed crocodilians (Foggin, 1992a; Foggin, 1992b; Buenviaje et al., 1994).

A probiotic is a preparation of microorganisms, used to restore or modify the composition of the microflora of a compartment of the body. Probiotics are increasingly being used in intensive animal production systems to improve growth rate and reduce disease incidence in farmed animals. The exact mechanisms by which probiotic bacteria affect the microecology of the intestinal tract are not well understood, but at least three mechanisms of action have been proposed: (i) production of antibacterial substances, (ii) modulation of immune responses and (iii) specific competition for adhesion receptors to intestinal epithelium (Nava et al., 2005). The effects of probiotics have not been studied in reptiles.

In contrast, the benefits of probiotics in intensive poultry, pig and calf rearing systems have been demonstrated. For example, Timmerman et al. (2005) found a reduced incidence of diarrhoea, decreased mortalities and improved growth rate in veal calves receiving probiotic supplementation. Zeyner and Boldt (2006) showed that daily intake of a probiotic containing *Enterococcus faecium,* from birth to weaning, reduced the portion of piglets suffering from diarrhoea, and led to increased weight gains. Piglets receiving a probiotic have also been shown to develop a more abundant GIT flora (Shim et al., 2005). The ability of probiotics to reduce incidence of specific diseases has been documented, for example coccidiosis in poultry (Dalloul et al., 2005), and *Lactococcus garvieae* and *Streptococcus iniae* infection in rainbow trout (Brunt and Austin, 2005).

Part 1 of this chapter is an investigation of the normal flora of the intestinal tract of wild Nile crocodiles in the Okavango Delta. Part 2 of this chapter is a pilot study regarding the development and application of a crocodile specific probiotic, based on the normal intestinal flora of wild Nile crocodiles. The purpose of this experiment was to determine if there was a beneficial effect, in terms of growth rate and survival, from artificially creating a mixed intestinal flora by supplementing farmed hatchlings with a probiotic.

**Study site**

Botswana's Okavango Delta, the world's biggest RAMSAR site, is a massive wetland within the Kalahari Desert, covering an area of approximately 16 000 $km^2$ in the dry season, and increasing to over 22 000 $km^2$ with the annual flood. The 111 250 $km^2$ active catchment area falls entirely within Angola. Due to the geology of the catchment, the incoming water is low in nutrients and sediment (Mendelsohn and Obeid, 2004).

The Okavango River flows through Namibia briefly before entering Botswana and forming a broad floodplain, the Panhandle. An estimated 40 % of incoming water leaks into the surrounding swamps by the time the river leaves the Panhandle. The remaining 60 % is distributed down three main channels, which fan out to form the Delta. The Okavango Delta consists of permanent and seasonal swamp, which is inundated during the annual flood.

The northern part of the Delta is characterized by shallow water, flooded grasslands, ox-bow lakes and lagoons mostly interconnected by narrow waterways. Only a few main channels lined by tall reeds (mainly *Phragmites australis*), carry the remainder of the Okavango's water southwards through the Delta. The permanent and seasonal swamp,

together form a unique ecosystem, providing high quality habitat for a great many species. As a keystone species, the Nile crocodile helps maintain the fragile balance within this ecosystem. Crocodiles are unevenly distributed throughout the delta, with the majority of the breeding population occurring in the 120 km long Panhandle.

The probiotic trial was carried out on the Krokovango crocodile farm at Samochima, Botswana.

**Study Animal**

The Nile crocodile, *Crocodylus niloticus*, is the most widespread and abundant of the three crocodile species that occur in Africa. It occurs throughout the continent south of the Sahara, in a variety of wetland habitats, including coastal areas (Taplin and Loveridge, 1988). Historically its distribution in southern Africa extended down the east coast as far as the Kei River.

The Nile crocodile is dark olive to brown, with a light yellow abdomen. Being reptiles, they are ectothermic and regulate their body temperature behaviourally, by moving between sun-exposed sandbanks and the water. Typical adult lengths are around 3.5 m, but they can grow up to 5 m (Groombridge, 1987). Sexual maturity is reached from 2.9 m total length for males, and 2.2 m for females (Cott, 1961). Nesting occurs in a hole in the ground, where an average of 50 eggs are laid. Nile crocodiles exhibit temperature-dependent sex determination (Leslie, 1997). Hatchlings emerge after an incubation period of approximately 90 days, in early to mid summer, and parental protection occurs (Pooley and Gans, 1976; Pooley, 1977). There is a very high mortality rate in their first year of life due to predation.

## Materials and Methods

### Part 1

### Capture method

Crocodiles were captured in the Panhandle of the Okavango during summer (February 2005). Capture was done at night, using a 4.8 m flat bottomed aluminium boat propelled by a 60 hp engine. Crocodiles were located using a 500 000 candle power spot-light which, once shone into the crocodile's eyes, reflected back a red glow due to the presence of a retinal tapetum lucidum. Once spotted, the beam of light was kept focused on the crocodile's eyes, making it possible to approach the animal with the boat. Crocodiles estimated to be smaller than 1.2 m total length (TL) were captured by hand. Crocodiles between 1.2 m and 2.3 m were captured using a swivelling noose (Animal Handling Co.) which was placed over the snout and pulled tight in the neck region. Crocodiles were then brought onto the boat, jaws were taped shut and the animals were physically restrained. Animals larger than 2.3 m were captured using a noose attached to a climbing rope, which was secured to the boat. The crocodile was allowed to swim so as to tire it out before it was brought onto the boat.

### Sampling

Twenty nine animals were randomly selected for cloacal swab collection. Each crocodile was blindfolded and restrained in dorsal recumbency. A cloacal swab was taken by inserting a sterile cotton swab (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, England) into the cloaca to the depth of 50 - 100 mm, rotating the swab, withdrawing it and placing it directly into the sterile transport medium supplied. Following blood collection the crocodile was measured: total length (TL) and snout-to-vent length (SVL) were recorded using a flexible measuring tape (±1 mm), and weighed using a harness around the forelimbs and a Pesola spring balance. Each crocodile was sexed by cloacal examination of the cliteropenis (Hutton, 1987; Leslie, 1997), and examined for clinical abnormalities including bite wounds, skin lesions, conjunctivitis, and poor condition. (Refer to Chapter 4).

**Isolation and identification procedures**

The cloacal swabs were stored at -10 °C in a domestic gas freezer for up to one month. On return from the study site, 29 swabs were submitted to the laboratory[2]. An aerobic bacterial culture and a fungal culture were performed as follows. Each cloacal swab was inoculated onto culture plates containing 5 % bovine blood and MacConkey agar no. 1 (Diagnostic Media Products, Sandringham, South Africa) and also on thiosulphate citrate bile salt sucrose (TCBS) agar, Mycosel agar, cornmeal agar and Rappaport Vassiliadis (RV) broth (the latter four all obtained from Selectamedia, Ferndale, South Africa).

Anaerobic culture could not be attempted, as many anaerobes would not have survived the storage process. All the agar plates were aerobically cultured at 25 °C, but the RV broth was cultured at 37 °C to improve selectivity for *Salmonella* isolation. The bacterial cultures were incubated for 72 hours before discarding, and the fungal cultures for 28 days before discarding. The RV broths were subcultured twice, after 24 hours and after 6 days of incubation, onto xylose lactose sodium desoxycholate (XLD) agar (Selectamedia). The XLD agars were cultured at 37 °C for 24 hours each time, and examined for the presence of colonies resembling *Salmonella*.

Each bacterial and fungal isolate was identified according to standard methods (Cowan, 1974; Campbell and Stewart, 1980; Krieg and Holt, 1984; Sneath et al., 1986; Rippon, 1988).

The bacterial strains that were isolated were lyophilized according to the working seed method (Balows et al., 1991). Each isolate was inoculated onto blood tryptose agar (BTA) plates (Selectamedia, Ferndale, RSA) to ensure pure cultures and incubated in a Gallenkamp incubator (Weiss Gallenkamp, UK) at 25 °C for 18-24 hours. After incubation culture purity was examined macroscopically by examining the colonies, and microscopically by examining a Gram smear (Balows et al., 1991). Each pure culture was then inoculated in 200 ml liquid broth Biolab tryptone soya broth (TSB) 30 g/l, and incubated at 25 °C in a shaking incubator. The purity of each 200 ml inoculum was validated. This was done by inoculation of 10 µl of each culture which was streaked on a blood agar plate. A Gram smear was subsequently made of the culture. Each inoculum was then centrifuged at 3000 G for 15 minutes, and re-suspended in 20 ml of phosphate buffered saline (PBS) with 50 % buffered lactose peptone (BLP). A 2 ml aliquot of each isolate was transferred into a 7 ml glass vial and lyophilized in a Virtis Genesis 25XL

[2] Golden Vet Lab, Johannesburg.

freeze dryer (Virtus, USA) at -20 °C for 24 hours, using the Virtis Wizard 2.0 software program. The glass vials were then sealed and labelled, and stored at 2-8 °C.

**Part 2**

**Probiotic production**

In February 2006, four of the bacterial strains were selected on the basis of their frequency of occurrence in wild crocodile intestinal tracts. These were *Enterococcus faecalis, Aeromonas hydrophila, Flavobacterium aquatile* and *Citrobacter koseri.* Each isolate was resuspended and the incubation process described above was repeated in 2 litre Chemap fermenters. The suspension of each isolate was titrated by plate counts and the growth curve determined via spectrophotometry.

After final concentration determination each isolate contained the following:

*E. faecalis* = $2.3 \times 10^9$ cfu/ml

*C. koseri* = $2.1 \times 10^9$ cfu/ml

*F. aquatile* = $2.0 \times 10^9$ cfu/ml

*A. hydrophila* = $1.8 \times 10^8$ cfu/ml

A 2 ml aliquot of each isolate was lyophilized as described above, in 7 ml glass vials, sealed and labelled.

**Experimental design**

The probiotic experiment was initiated during the fourth week of February 2006, on hatchlings on the Krokovango crocodile farm. One hundred one-month old farmed hatchlings were divided equally into a control and treatment group. Each of the four organisms were reconstituted with sterile PBS and mixed, to provide a total volume of 100 ml. The hatchlings in the treatment group were dosed with 2 ml of probiotic on the first day, by passing a 1 ml insulin syringe into the oesophagus twice. Each hatchling was weighed and permanently identified by excising certain tail scutes in a coded sequence (Leslie, 1997). The controls received the same treatment, but, were dosed with sterile water. The following day the treatment was repeated, except that the probiotic excluded the *C. koseri.* This was unintentional: the second *C. koseri* vial was unfortunately destroyed during transport.

The two groups were then kept in two separate rearing pens, in the farm's hatchling hothouse. All further management was according to the farm's usual routine.

Approximately half of the area of each 7.5 m² tiled rearing pen consisted of a shallow water bath. The water temperature in the baths was generally maintained between 29-31 °C. Heating was accomplished by running hot water through the submerged heating pipes. An exception occurred between June and August when the water temperature in the early morning dropped to approximately 26 °C. The hothouse was not completely enclosed, and the air temperature in the house could not be controlled.

The water was drained daily, and the pens cleaned. The surface was sprayed with F10 (Health and Hygiene, South Africa) before the baths were re-filled with water pumped from the Okavango River. No disinfectants were added to the water. Nor did the hatchlings receive any antibiotic treatments.

After three months the two groups were mixed together in one rearing pen.

The hatchlings were fed daily, *ad lib* with a diet consisting of 50 % marine fish and 50 % "concentrate", where the concentrate was formulated as follows:

Fish-meal: 50 %

Carcass-meal: 36.5 %

Precooked maize: 8 %

Bone-meal: 2 %

Trace element premix (Feedmix Pty Ltd, South Africa): 2 %

Vitamin and amino acid supplement (Antec International): 1.5 %

Hatchlings were weighed five times at varying intervals over the following five and a half months. At the final weighing eight hatchlings were randomly selected and cloacal swabs collected from them. The swabs were placed on ice and submitted to the laboratory[3] within 48 hours for aerobic bacterial culture.

**Isolation and identification procedures**

Each cloacal swab was inoculated onto culture plates containing 5 % equine blood, MacConkey agar no. 1 and Rappaport Vassiliadis (RV) broth (Selectamedia). All the agar plates were aerobically cultured at 25 °C, but the RV broth was cultured at 37 °C to improve selectivity for *Salmonella* isolation. The bacterial cultures were incubated for 72 hours before discarding. The RV broths were subcultured after 24 hours onto XLD agar

[3] Gloden Vet Lab, Johannesburg.

(Selectamedia). The XLD agars were cultured at 37 °C for 24 hours and examined for the presence of colonies resembling *Salmonella*.

Each bacterial isolate was identified according to standard methods. (Cowan, 1974; Campbell and Stewart, 1980; Krieg and Holt, 1984; Sneath et al., 1986; Rippon, 1988).

The *Salmonella* isolates were submitted to the Onderstepoort Veterinary Institute for typing.

**Statistical analysis**

The mass gains of the treatment and control groups were analyzed for significant difference by one way analysis of variance. The residuals were checked for normality of distribution with normal probability plots. If the residuals were found not to be normally distributed, significance was then tested by a Mann-Whitney test. Difference in mortality rate between the two groups was analyzed by the maximum-likelihood Chi-square test.

**Results**

**Part 1**

The bacteria and fungi isolated from each wild specimen are presented in Table 3.1. In this table the bacteria and fungi are given in the order of frequency in which they were isolated from each cloacal swab, with the first named being the most frequent isolate.

(i) Bacteria were cultured from all 29 specimens. There were a total of 79 isolations, and 16 different species. The number of species cultured per specimen varied from one to four, with only two (6.9 %) specimens yielding a single species that could be cultured under the incubation conditions described. Eight crocodiles (27.6 %) had two isolates and 15 (51.7 %) yielded three isolates. Four isolates were obtained from four crocodiles (13.8 %). The mean number of isolations per crocodile was 2.7.

Table 3.2 shows the number of isolates of each bacterium, and the percentage of crocodiles carrying each species. The most commonly isolated species was *Microbacterium*, found in 21 of the crocodiles (72.4 %), followed by *Enterococcus faecalis* (14 isolates), *Aeromonas hydrophila* (10 isolates), and *Escherichia coli* (nine isolates). No salmonellae were cultured.

Table 3.1: Bacterial and fungal isolates from each cloacal swab from the 29 wild caught Nile crocodiles that were sampled.

| Animal no. | Bacterial isolates | Fungal isolates |
|---|---|---|
| 1 | *Enterococcus faecalis, Escherichia coli, Microbacterium, Proteus vulgaris* | none |
| 2 | *Aeromonas hydrophila, Microbacterium* | none |
| 3 | *Cytophaga succinicans, Flavobacterium aquatile, Microbacterium* | none |
| 4 | *Enterococcus faecalis, Pseudomonas stutzeri, Microbacterium* | *Penicillium* |
| 5 | *Enterococcus faecalis, Microbacterium, Staphylococcus epidermidis, Flavobacterium aquatile* | *Chrysosporium* |
| 6 | *Escherichia coli, Enterococcus faecalis, Microbacterium* | *Cladosporium* |
| 7 | *Escherichia coli, Enterococcus faecalis, Microbacterium* | none |
| 8 | *Escherichia coli, Staphylococcus epidermidis, Microbacterium* | none |
| 9 | *Escherichia coli, Enterococcus faecalis, Microbacterium, Enterobacter intermedium* | none |
| 10 | *Enterobacter intermedium, Enterococcus faecalis, Microbacterium* | none |
| 11 | *Aeromonas hydrophila, Flavobacterium aquatile, Cytophaga heparina* | *Cladosporium* |
| 12 | *Enterococcus faecalis, Microbacterium, Cytophaga heparina* | *Cladosporium* |
| 13 | *Aeromonas hydrophila* | *Cladosporium* |
| 14 | *Escherichia coli, Microbacterium* | *Cladosporium, Penicillium* |
| 15 | *Escherichia coli, Enterococcus faecalis, Aeromonas hydrophila* | *Cladosporium* |
| 16 | *Microbacterium, Citrobacter koseri, Enterococcus faecalis* | *Cladosporium* |
| 17 | *Microbacterium, Enterococcus faecalis, Escherichia coli* | *Trichoderma* |
| 18 | *Microbacterium, Aeromonas hydrophila* | *Cladosporium* |
| 19 | *Citrobacter koseri, Enterococcus faecalis, Microbacterium, Aeromonas hydrophila* | *Chrysosporium* |
| 20 | *Aeromonas hydrophila, Microbacterium* | none |
| 21 | *Enterococcus faecalis, Aeromonas caviae* | none |
| 22 | *Proteus mirabilis, Microbacterium, Enterococcus faecalis* | *Trichoderma* |
| 23 | *Aeromonas hydrophila, Microbacterium, Flavobacterium aquatile* | none |
| 24 | *Microbacterium, Citrobacter koseri* | none |
| 25 | *Bacillus subtilus, Aeromonas hydrophila, Cytophaga heparina* | *Exophiala, Saprolegnia* |
| 26 | *Flavobacterium aquatile, Microbacterium, Escherichia coli* | none |
| 27 | *Enterococcus avium* | none |
| 28 | *Aeromonas hydrophila, Cytophaga heparina* | none |
| 29 | *Cytophaga heparina, Citrobacter koseri* | none |

Table 3.2: Number of isolates of each bacterium, and the percentage of wild crocodiles carrying each species.

| Bacteria | No of isolates | Percentage of crocodiles |
|---|---|---|
| *Aeromonas caviae* | 2 | 6.9 % |
| *Aeromonas hydrophila* | 10 | 34.5 % |
| *Bacillus subtilus* | 1 | 3.4 % |
| *Citrobacter koseri* | 4 | 13.8 % |
| *Cytophaga heparina* | 4 | 13.8 % |
| *Cytophaga succinicans* | 1 | 3.4 % |
| *Escherichia coli* | 9 | 31.0 % |
| *Enterobacter intermedium* | 2 | 6.9 % |
| *Enterococcus avium* | 1 | 3.4 % |
| *Enterococcus faecalis* | 14 | 48.3 % |
| *Flavobacterium aquatile* | 5 | 17.2 % |
| *Microbacterium* | 21 | 72.4 % |
| *Proteus mirabilis* | 1 | 3.4 % |
| *Proteus vulgaris* | 1 | 3.4 % |
| *Pseudomonas stutzeri* | 1 | 3.4 % |
| *Staphylococcus epidermidis* | 2 | 6.9 % |

(ii) Fungi were isolated from 14 of the 29 crocodiles (48.3 %). There were 16 isolations, of six different species. Twelve crocodiles (41.4 %) yielded a single species, while just two crocodiles (6.9 %) yielded two species.

Table 3.3 shows the number of isolates of each fungus, and the percentage of crocodiles carrying each fungus. The most commonly isolated species was *Cladosporium*, found in eight crocodiles (27.6 %).

Table 3.3: Number of isolates of each fungus, and the percentage of crocodiles carrying each fungus.

| Fungi | No of isolates | Percentage of crocodiles |
|---|---|---|
| *Chrysosporium* | 2 | 6.9% |
| *Cladosporium* | 8 | 27.6% |
| *Exophiala* | 1 | 3.4% |
| *Penicillium* | 2 | 6.9% |
| *Saprolegnia* | 1 | 3.4% |
| *Trichoderma* | 2 | 6.9% |

**Part 2**

The mean crocodile mass and cumulative mortality rates at each weighing date are presented in Table 3.4, for both the treatment and control groups.

**Growth rate**

The growth rates of individual crocodiles in both groups were extremely variable. Over the 5.5 month period, the total mass gain of the treatment hatchlings varied from 6 g - 806 g, and the controls varied from 1 g - 632 g.

The mean mass at the beginning of the experiment was essentially the same in both groups (treatment group: 76.7 g; control group: 76.6 g). Mean mass gain between the groups remained similar for the first 5 weeks (treatment group 18.3 g; controls 18.6 g). Over the following six weeks the treatment group grew more slowly, gaining an average of 51.6 g, while the controls gained an average of 95.0 g (Figure 3.1). During the next six weeks the growth rates were similar. However, the treatment group still grew slightly slower, gaining an average of 147.8 g, while the controls gained an average of 154.7 g. During the final two weeks of the experiment the mean mass of both groups decreased. The treatment group decreased by 5.1 g, while the control group decreased by 8.5 g.

The final mean mass gain of the surviving crocodiles in the treatment group was 210.9 g, compared to 257.8 g for the controls. The surviving crocodiles in the treatment group ended with a mean mass of 289.3 g, 14 % lighter than the controls at 336.4 g.

Table 3.4: Mean hatchling mass and cumulative mortality rate in treatment and control groups, at each weighing date.

| | Treatment Group | | | Control Group | | |
|---|---|---|---|---|---|---|
| Date | n | Mean mass ± SD (g) | Cumulative mortality rate (%) | n | Mean mass ± SD (g) | Cumulative mortality rate (%) |
| **22-Feb** | 50 | 76.7 ± 8.9 | 0 | 50 | 76.6 ± 7.7 | 0 |
| **27-Feb** | 50 | 79.1 ± 10.5 | 0 | 50 | 75.7 ± 11.0 | 0 |
| **28-Mar** | 50 | 95.0 ± 43.0 | 0 | 50 | 95.2 ± 41.5 | 0 |
| **12-May** | 50 | 146.6 ± 100.8 | 0 | 49 | 190.2 ± 133.3 | 2 |
| **24-Jul** | 36 | 294.4 ± 246.7 | 28 | 37 | 344.9 ± 200.9 | 26 |
| **6-Aug** | 35 | 289.3 ± 242.4 | 30 | 32 | 336.4 ± 187.6 | 36 |

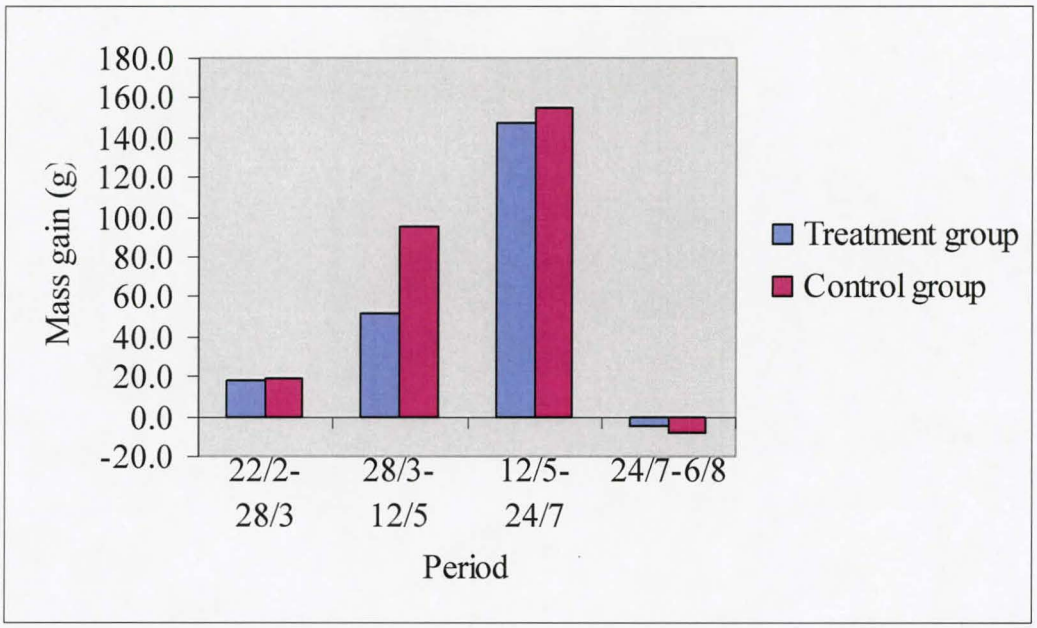


Figure 3.1: Mean mass gain of treatment and control hatchlings during each time period.

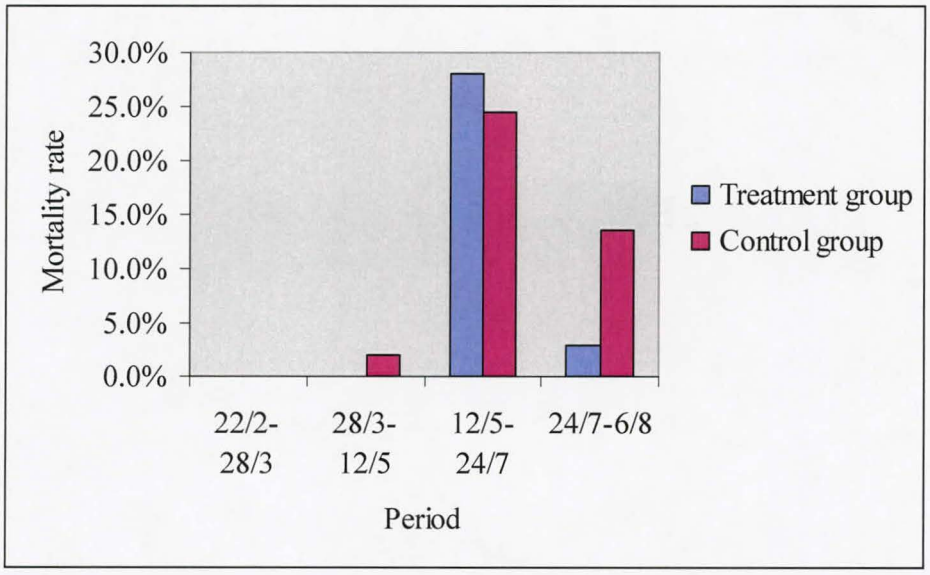


Figure 3.2: Mortality rate of treatment and control hatchlings during each time period.

**Mortality rate**

The survival rate over the first 11 weeks was excellent, with the loss of only one control crocodile. However, over the following six weeks 14 out of 50 hatchlings in the treatment group, and 12 of 49 in the control group died, representing a mortality rate of 28 % and 24.5 % respectively. Over the final two weeks one of the remaining 36 treatment

hatchlings died, a mortality rate of 2.8 %. During the same period, five of the remaining 37 control hatchlings died, a mortality rate of 13.5 %.

The cumulative mortality rates for the entire period were 30 % for the treatment group and 36 % for the controls. In comparison, the farm's average hatchling mortality rate for the season up to 06 August 2006 was 10 %.

**Intestinal flora**

The results of the bacterial cultures from the trial hatchlings are shown in Table 3.5 and 3.6. Of the four species included in the probiotic, only two, *E. faecalis* and *A. hydrophila* were cultured. A heavy mixed growth was isolated from all eight samples. Some of the more slowly growing bacteria may therefore have been overgrown, such as *F. aquatile*. *Enterococcus faecalis* and *A. hydrophila* were cultured from all eight samples, there being no significant difference between the control and treatment groups. The *E. faecalis* and *A. hydrophila* are possibly the same strains that were in the probiotic, but as they are very common in the environment of crocodiles, they may be different strains.

Table 3.5: Cloacal isolates from three hatchlings treated with probiotic.

| Hatchling ID | *Aeromonas hydrophila* | *Morganella morganii* | *Acinetobacter* | *Psychrobacter* | *Escherichia coli* | *Enterococcus faecalis* | *Proteus* | *Salmonella* | Total isolates |
|---|---|---|---|---|---|---|---|---|---|
| R3L3S2 | + | - | - | - | - | + | + | + 07:d:(1,2) | 4 |
| R3L4S7 | + | + | + | + | + | + | + | - | 7 |
| R1L8S7 | + | - | - | + | + | + | + | + 0 polyvalent | 6 |
| Mean | | | | | | | | | 5.7 |

The number of species cultured from the treatment hatchlings varied from four to seven with a mean of 5.7 species. The number of species cultured from the control hatchlings varied from five to seven with a mean of 6.2 species. *Salmonella* was cultured from seven of the eight swabs, a prevalence of 87.5 %. *Salmonella* was cultured from three out of four (75 %) of the treatment group, and five out of five (100 %) from the control group. The

*Salmonella* strains that were isolated, were biochemically identified as *Salmonella enterica* subspecies *salamae* (Popoff and Le Minor, 2005) which corresponds to/with the previously recognized group II (Krieg and Holt, 1984). Two of the isolates agglutinated with a *Salmonella* polyvalent antiserum (Biorad), but did not react with the polyvalent A and B sera, indicating that those strains probably belonged to rarely isolated serovars. No other polyvalent sera were available. Five of the isolates were positive for agglutination with the O 7 agglutinating serum. Three of these isolates were further typed using H typing sera[4]. These three were all positive for b, but no further typing could be carried out, due to the non-availability of z39 and z42 typing sera. According to the Kauffmann-White scheme of *Salmonella* serovars listed in Popoff and Le Minor (2005), the three strains could only have been either *Salmonella* II 6,7:b:[enx]:z42 or *Salmonella* II 6,7:b:z39. *Salmonella* serovars belonging to groups other than group I (*Salmonella enterica* subspecies *enterica*) are not named, but are known by their antigenic formula.

Table 3.6: Cloacal isolates from five control hatchlings.

| Hatchling ID | *Aeromonas hydrophila* | *Morganella morganii* | *Acinetobacter* | *Psychrobacter* | *Escherichia coli* | *Enterococcus faecalis* | *Proteus* | *Salmonella* | Total isolates |
|---|---|---|---|---|---|---|---|---|---|
| R1L4S4 | + | + | + | - | - | + | - | + 07 | 5 |
| R1L6S1 | + | - | + | - | + | + | + | + 07 | 6 |
| R1L6S5 | + | - | + | + | + | + | - | + 07 | 6 |
| R3L6S5 | + | + | + | - | + | + | + | + 0 polyvalent | 7 |
| R3L8S8 | + | - | + | + | + | + | + | + 07 | 7 |
| Mean | | | | | | | | | 6.2 |

[4] At Onderstepoort Veterinary Institute.

## Discussion

### Part 1

The bacterial species most commonly isolated from the wild Nile crocodiles, *Microbacterium*, is a common soil inhabitant. Neither *Microbacterium* nor *E. faecalis*, the second most frequently isolated species, are associated with bacterial septicaemia in crocodiles. Misra et al. (1993) did not isolate *Microbacterium* or *Enterococcus* from cloacal swabs from 23 gharials, nor was *Microbacterium* isolated from the intestinal contents of 29 African dwarf crocodiles (Huchzermeyer et al., 2000). *Enterococcus faecalis* was, however, isolated from one of the dwarf crocodiles, and other *Enterococcus* species were isolated from a further 21 of the 29 dwarf crocodiles.

*Escherichia coli* appears to be a common component in crocodile intestinal tract flora, having also been isolated from nine of the gharials and eight of the dwarf crocodiles. Nevertheless *E. coli* has been recorded as a cause of septicaemia in crocodilians, including the Nile crocodile. Foggin (1992b) found 47 out of 409 (11.5 %) bacterial infections to be caused by *E. coli.*

*Aeromonas hydrophila* was isolated from 34.5 % of the samples. *Aeromonas hydrophila* is frequently found associated with mortality caused by enteritis and septicaemia. In Zimbabwe it was the second most frequent isolate, after *Salmonella*, from septicaemic Nile crocodiles (Foggin, 1992b). It is also an important cause of septicaemia in *Crocodylus porosus, Crocodylus johnsoni* and *Crocodylus novaeguineae* (Buenviaje et al., 1994; Ladds and Simms, 1990).

Besides *E. coli* and *A. hydrophila* another five of the genera isolated (*Bacillus, Citrobacter, Proteus, Pseudomonas and Staphylococcus*) are known causes of septicaemia in crocodiles (reviewed by Huchzermeyer, 2003). This supports the view that many bacterial septicaemias are caused by normal intestinal tract inhabitants which act as opportunistic pathogens in an immunosupressed host.

No *Salmonella* were cultured from the wild Nile crocodiles. This is interesting in the light of previous findings. Of 67 wild Nile crocodiles in Lake Kariba, 18 (26.9 %) grew *Salmonella* (Madsen et al., 1998). Three of 29 (10.3 %) wild caught African dwarf crocodiles yielded *Salmonella* (Huchzermeyer et al., 2000). Scott et al (1997) found *Salmonella* in 2 of 71 (2.8 %) wild American alligators (*Alligator mississippiensis*).

In farmed crocodilians, *Salmonella* has frequently been isolated: Obwolo and Zwart (1993) found *Salmonella* in 8 out of 50 healthy three year old farmed Nile crocodiles, and

concluded that *Salmonella* may represent normal flora in the intestinal tract of crocodiles. In contrast Misra et al. (1993) did not find any *Salmonella* in cloacal swabs from 23 captive gharials. Healthy farmed *C. porosus* and *C. johnstoni* were found to carry *Salmonella* (Manolis et al., 1991). On one farm 20.0 % of *C. porosus* and 27.8 % of *C. johnstoni* were carriers, while on another farm the prevelance rate was 81 % and 5 % for the 2 species respectively. Scott and Foster (1997) found *Salmonella* in 4 of 29 (14 %) farmed alligators.

In contrast to their frequent occurrence as resident flora, *Salmonella* was cultured from 202 out of 409 farmed Nile crocodiles found to have died from bacterial infections in Zimbabwe (Foggin, 1992b). Van der Walt et al (1997) reported 145 *Salmonella* isolates and a wide range of serovars from farmed Nile crocodiles submitted for necropsy over a 10 year period.

It is clear that while *Salmonella* can be normal intestinal tract flora in healthy farmed crocodilians, they can also be important pathogens. The role of *Salmonella* as normal intestinal tract flora in wild crocodilians is unclear. DuPont et al. (1978) found that *Salmonella* could suddenly be excreted from turtles after a period of 6 months with no excretion. Furthermore, cloacal swabbing may underestimate the prevalence of *Salmonella*, compared to faecal swabbing (Manolis et al., 1991). The composition of intestinal flora is dependant on ingested food, both the type of food and the amount. These may be factors in the apparent absence of *Salmonella* in this study. Logically there will be less efficient horizontal transfer of *Salmonella* in a natural environment than under intensive conditions. Nevertheless, vertical transmission could occur with equal ease in either environment. Recent findings from *C. porosus* eggs tend to support the possibility of vertical transmission of *Salmonella*. *Salmonella* was cultured in eggs from 12 out of 13 clutches on one farm. Interestingly, the serotypes isolated were clutch specific (Peuker et al., 2005).

The fungi isolated from the wild Nile crocodiles are considered environmental. Nevertheless, *Cladosporium, Penicillium* and *Trichoderma* have been found in diseased crocodilians (Troiano and Roman, 1996; Buenviaje et al., 1994; Hibberd et al., 1996; Foreyt et al., 1985). These three fungi have also been isolated from the shells of *C. porosus* eggs, but were not present in the egg yolk (Peuker et al., 2005).

Huchzermeyer et al. (2000) isolated fungi in the intestinal tract of 24 out of 29 African Dwarf crocodiles, a far higher occurrence than the present study.

**Part 2**

The probiotic did not result in an increased growth rate, nor was the mortality rate meaningfully reduced. Furthermore the mortality rate of both the control and treatment groups was much higher than the farm's average, indicating that there was likely a management-related factor involved.

The aerobic bacterial cultures from the cloacal swabs collected from the farmed hatchlings yielded the same eight bacterial species from the trial and control groups. The probiotic did not alter the composition of the intestinal tract flora, nor the average number of species per crocodile. Four of the eight bacterial species isolated have previously been associated with septicaemia in Nile crocodiles: *A. hydrophila, M. morganii*, *E. coli* and *Salmonella* (Foggin, 1987 and 1992a; Van der Walt et al., 1997), and *Proteus* sp. has been isolated from other septicaemic crocodilians (Novak and Seigel, 1986; Chakraborty et al., 1988).
Although the total range of species was narrower than those isolated from the wild crocodiles, the average number of species carried per crocodile was greater (5.7 and 6.2 compared to 2.7 from the wild crocodiles). Clearly these hatchlings were capable of establishing a diverse resident intestinal tract flora by the age of 6.5 months. The fact that there were no antibiotics in the feed and no disinfectants in the pond water probably contributed to this. Nevertheless, we cannot say how early the intestinal tract flora became established. Future probiotic trials should include intestinal tract cultures at regular intervals from the outset.
*Salmonella* was cultured from seven of the eight swabs (87.5 %). This is a very high incidence, but correlates with the prevalence of over 80 % found in farmed crocodiles in Zimbabwe (Madsen et al., 1998). The isolates were all *Salmonella enterica* subspecies *salamae* which are frequently found in cold-blooded animals and the environment (Popoff and Le Minor, 2005). Subspecies of *Salmonella* more commonly found to be pathogenic in mammals usually belong to *Salmonella enterica* subspecies *enterica.*
The dramatic difference in *Salmonella* prevalence in the farmed hatchlings compared to the wild crocodiles indicates that *Salmonella* is much more efficiently transmitted in an artificially intensive environment. These hatchlings were not fed poultry, often suspected of being the source of *Salmonella* in young crocodiles.

The finding that the probiotic produced no beneficial effect is surprising in light of the widespread use of probiotics in other species, particularly other recently domesticated

stress-prone species such as the ostrich (Huchzermeyer, 1994). In the aquaculture industry, beneficial effects of probiotics have been demonstrated in several species, for example rainbow trout (Brunt and Austin, 2005), Atlantic salmon (Austin et al., 1992), crabs (Nogami and Meada, 1992), prawns (Maeda and Liao, 1992) and oysters (Douillet and Langdon, 1994).

Several factors may have influenced these results:

These hatchlings were simultaneously used in another trial, investigating temperature dependant sex determination. The eggs were artificially incubated at eight different temperatures (30.0 °C – 34.5 °C). Following hatching the crocodiles were handled several times for the purposes of identification, weighing and measuring, and sex determination. It is well established that handling is a significant stressor of crocodilians, resulting in increased plasma corticosterone concentrations, and altered leukocyte counts, with lymphocytopaenia by 24 hours post exposure (Lance and Elsey, 1986; Elsey et al., 1991). In an experiment to test the effect of high density rearing (Elsey et al., 1989) plasma corticosterone and growth rate showed a strong negative correlation. Lance (1990) concludes that crocodilians respond to non-specific stress with a chronic increase in corticosterone secretion, an inhibition of growth, an inhibition of the reproductive system and a suppression of the immune system.

A cold winter during the second half of the experimental period resulted in lower temperatures in the rearing house than was ideal. To counteract the low air temperatures, rearing pens were frequently completely flooded at night, with water temperature reported at approximately 26 °C the following morning. This is well below the ideal of approximately 32 °C (Foggin, 1992a). Temperature has long been known to play an important role in reptile immunity (Metchnikoff, 1901). Lance and Elsey (1999) demonstrated increased plasma corticosterone and altered white cell counts after exposure of American alligators to cold shock. Cold shock is known to be associated with increased susceptibility to disease in reptiles. While the experimental hatchlings would not have suffered cold shock at 26 °C, it was colder than the temperature that they had been adapted to in captivity, and as such would have been a source of stress.

From the above it is evident that stress associated with repeat handling and sub-optimal temperature may have altered growth rates as well as disease susceptibility, with associated altered mortality rates.

In the final weeks of the experiment up to 40 % of the hatchlings were suffering from pox virus infection, characterized by brown spots of 1-2 mm diameter, distributed mainly over the skin of the ventral abdomen. Some hatchlings also had large cutaneous erosions of up to 10 mm on the tail. This condition was also noted in other hatchling groups on the farm, but at a lower incidence than in the experimental hatchlings. Pox virus infection has been reported to be associated with reduced growth rates and increased mortalities (Foggin, 1987, Pandey et al., 1990). It is likely therefore that the growth and survival of hatchlings in this trial were affected by the presence of this disease. Furthermore, some of the tail erosions affected the tail scutes, making identification of a few individuals difficult. Some errors may have resulted from this.

The bacterial species included in the probiotic were selected on the basis of frequency of isolation from intestinal tract cultures obtained from wild Nile crocodiles. However, the two most frequently occurring species (*Microbacterium* and *E. coli*) did not survive the freeze drying process and therefore could not be included. For the same reason none of the fungal species isolated were included. As there has been no work done in this field, it is difficult to know which of the normal intestinal tract flora is the most beneficial species. Many more trials will be required to test the effect of various different species when included in a probiotic. Anaerobes would be an important additional consideration.

The hatchlings were one month old at the start of the experiment. This may have been too late to achieve the objective of rapidly establishing mixed bacterial communities in the intestinal tract, essential for the prevention of colonization by pathogenic bacteria. Future probiotic trials in crocodiles should consider starting with day old hatchlings. Furthermore the hatchlings should be swabbed before the commencement of the trial to establish the baseline intestinal flora.

In this trial the probiotic was dosed twice, on consecutive days. In many other species probiotics are included in the feed and therefore there is continuous intake. This may be a better approach with crocodiles particularly, as it obviates the need to handle the hatchlings, which will reduce stress.

River water and fresh fish are potential sources of the same bacteria which were found in crocodile intestinal tracts. Future probiotic trials should be designed such that the experimental hatchlings are kept in borehole water instead of river water, and fresh fish should not form part of the diet.

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# CHAPTER 4

## A Disease Survey in the Wild Nile Crocodile (*Crocodylus niloticus*) Population in the Okavango Delta, Botswana

### Introduction

Since the development of the crocodile farming industry in the 1980's, a lot of research has been carried out worldwide on diseases in farmed crocodiles (Foggin, 1987 and 1992b; Ladds et al., 1990 and 1995; Buenviaje et al., 1994 and 1998; Huchzermeyer, 2002). Very little, however, is known about the diseases of wild crocodilians, including wild Nile crocodiles. Wild crocodiles are often difficult to study due to the remoteness of the areas in which they occur.

The initial objective of this study was to determine disease prevalence in the wild Nile crocodile population in the Okavango Delta. However, several factors made this difficult:

(i) Very few serological tests have been developed for crocodile diseases. Consequently a serological survey is of very limited value.

(ii) Virus isolation is impossible due to the lack of available crocodile cell lines. The crocodile viruses cannot be grown in embryonated chicken eggs, or any of the cell culture lines available in veterinary diagnostic laboratories.

(iii) Sick crocodiles are difficult to identify by observation. Sick crocodiles under farmed conditions often stop eating but show no other clinical signs, and as such are not noticed as unhealthy until they are found dead. Diseases causing visible external lesions, for example pox virus, are the obvious exception.

(iv) Clinical examination has several limitations: cardiac and respiratory rates are variable according to the conditions, and are severely influenced by capture stress. Due to their poikilothermic nature, body temperature cannot be used as a diagnostic tool.

(v) Due to the predatory environment in which they live, sick/weak crocodiles are unlikely to survive long. Therefore, the likelihood of encountering these individuals in a capture and release survey is low.

(vi) No crocodiles could be sacrificed for histopathological and parasitological examination.

Considering these limitations, it was postulated that we would find a low incidence of diseases in the Okavango population, during a capture and release survey.

However, in the case of mycoplasmosis and heamogregarine infection it was possible to do a more accurate survey, due to the availability of diagnostic tests:

(i) Mycoplasmosis

The first recorded outbreak of mycoplasmosis in crocodiles occurred in Zimbabwe on five farms simultaneously (Mohan et al., 1995). Rearing stock 1-3 years of age developed swollen limb joints and lameness. Morbidity was 10 % and mortality even lower. A new species of mycoplasma was cultured from the joints of affected animals. The disease was then reproduced in healthy crocodiles by experimental infection with this isolate. Kirchoff et al. (1997) assigned this mycoplasma to a new species: *Mycoplasma crocodyli*.

Also in 1995 a highly fatal outbreak of disease, characterized by arthritis and pneumonia, occurred in farmed alligators (*Alligator mississippiensis*) in Florida (Brown et al., 1996). Nine out of 74 adult animals died over a 10 day period. A new species of mycoplasma was isolated. Losses continued over the next six months, despite tetracycline treatment, until only 14 alligators remained. This species was later assigned as *M. alligatoris* (Brown et al., 2001a). An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies produced by alligators in response to *M. alligatoris* exposure has been developed (Brown et al., 2001b).

Following the initial outbreak in Zimbabwe, cases of mycoplasmosis were reported year after year, from about one third of Zimbabwean crocodile farms. An autogenous vaccine was developed which, in an experimental trial, afforded limited protection (Mohan et al., 1997). Then a severe outbreak occurred in crocodiles following movement, in which the morbidity was over 50 % and the mortality rate over 20 % (Mohan et al., 2001). Subsequently mycoplasmosis has become an important disease on South African crocodile farms, with several large outbreaks having occurred (Picard, pers comm.).

The epidemiology of this disease is not well understood. The source of the original Zimbabwean outbreak remains elusive. Huchzermeyer (2003) suggested that wild crocodiles may act as reservoirs of infection, with vertical transmission being the main mode of transmission. However, unidentified mycoplasmas have been found in the faeces of farmed Nile crocodiles, indicating the possibility of horizontal transmission (Huchzermeyer et al., 1994). The concept of wild crocodilian reservoirs of infection has recently been confirmed in the case of *M. alligatoris.* In a seroprevalence study, Brown et al. (2005) found 5.4 % of wild American alligators positive for *M. alligatoris* antibodies, at

12 of 20 sites (60 %). Further elucidation of the epidemiology of mycoplasmosis in Nile crocodiles requires that the mycoplasma status of wild crocodiles be established.

Many crocodile farms collect eggs from wild crocodile nests. If the wild population concerned is infected then this is a very risky practice. Often these farms are obliged to re-introduce a certain number of juvenile individuals into the wild. If, on the other hand, the wild population is free from mycoplasmosis, it could be disastrous to reintroduce exposed juveniles.

(ii) Heamogregarines

Haemogregarines are protozoal blood parasites. Members of the genus *Hepatozoon* occur in various crocodilians. Asexual shizonts are found in the liver of infected crocodiles and gametocytes are found in erythrocytes or free in the blood. Sexual multiplication occurs in the intermediate host, usually hematophagous insects (Lainson et al., 2003; Khan et al., 1980). Leeches may also be intermediate hosts, but transmission by leeches has not been proven (Khan et al., 1980).

*Hepatozoon pettiti* was described in Nile crocodiles by Thiroux (1910), in Senegal and Uganda. Then *H. sheppardi* was described from Nile crocodiles in Mozambique (Santos Dias, 1952). Recently Gomersall et al. (2006) confirmed that the species found in crocodiles in the Okavango is *H. pettiti*. The intermediate host of *H. pettiti* has not been determined. Hepatozoon parasites are thought to be apathogenic in their crocodilian hosts. In this survey the aim was to determine the prevalence of *H. pettiti* and its effect on the crocodile host.

**Study site**

Botswana's Okavango Delta, the world's biggest RAMSAR site, is a large wetland within the Kalahari Desert, covering an area of approximately 16 000 $km^2$ in the dry season, and increasing to over 22 000 $km^2$ with the annual flood. The 111 250 $km^2$ active catchment area falls entirely within Angola. Due to the geology of the catchment, the incoming water is low in nutrients and sediment (Mendelsohn and el Obeid, 2004).

The Okavango River flows through Namibia briefly before entering Botswana and forms a broad floodplain, the Panhandle. An estimated 40 % of incoming water leaks into the surrounding swamps by the time the river leaves the Panhandle. The remaining 60 % is distributed down three main channels, which fan out to form the Delta. The Okavango

Delta consists of permanent and seasonal swamp, which is inundated during the annual flood.

The northern part of the Delta is characterized by shallow water, flooded grasslands, ox-bow lakes and lagoons mostly interconnected by narrow waterways. Only a few main channels lined by tall reeds (mainly *Phragmites australis*), carry the remainder of the Okavango's water southwards through the Delta. The permanent and seasonal swamp, together form a unique ecosystem, providing high quality habitat for a great many species. As a keystone species, the Nile crocodile helps maintain the fragile balance within this ecosystem. Crocodiles are unevenly distributed throughout the delta, with the majority of the breeding population occurring in the 120 km long Panhandle.

**Study Animal**

The Nile crocodile, *Crocodylus niloticus*, is the most widespread and abundant of the three crocodile species that occur in Africa. It occurs throughout the continent south of the Sahara, in a variety of wetland habitats, including coastal areas (Taplin and Loveridge, 1988). Historically its distribution in southern Africa extended down the east coast as far as the Kei River.

The Nile crocodile is dark olive to brown, with a light yellow abdomen. Being reptiles, they are ectothermic and regulate their body temperature behaviourally, by moving between sun-exposed sandbanks and the water. Typical adult lengths are around 3.5 m, but they can grow up to 5 m (Groombridge, 1987). Sexual maturity is reached from 2.9 m total length for males, and 2.2 m for females (Cott, 1961). Nesting occurs in a hole in the ground, where an average of 50 eggs are laid. Nile crocodiles exhibit temperature-dependent sex determination (Leslie, 1997). Hatchlings emerge after an incubation period of approximately 90 days, in early to mid summer, and parental protection occurs (Pooley and Gans, 1976; Pooley, 1977). There is a very high mortality rate in their first year of life due to predation.

## Materials and Methods

### Capture method

One hundred and forty four crocodiles were captured in the Panhandle of the Okavango during summer (February 2005). Capture was carried out using two methods: (i) at night, using a 4.8 m flat bottomed aluminium boat propelled by a 60 hp engine. Crocodiles were located using a 500 000 candle power spot-light which, once shone into the crocodile's eyes, reflected back a red glow due to the presence of a retinal tapetum lucidum. Once spotted, the beam of light was kept focused on the crocodile's eyes, making it possible to approach the animal by boat. Crocodiles estimated smaller than 1.2 m total length (TL) were captured by hand. Crocodiles between 1.2 m and 2.3 m were captured using a swivelling noose (Animal Handling Co.) which was placed over the snout and pulled tight in the neck region. Crocodiles were then brought onto the boat, jaws were taped shut and the animals were physically restrained. Animals larger than 2.3 m were captured using a noose attached to a climbing rope, which was secured to the boat. The crocodile was allowed to swim so as to tire it out before it was brought onto the boat. (ii) Traps: baited box and Pitman traps were strategically placed on river banks. Traps were baited at sunset and checked at first light the next morning. Captured animals were immediately restrained and the necessary data collected.

### Crocodile processing

Each crocodile was blindfolded and restrained in ventral recumbency. Fifty three animals were randomly selected for blood collection. Blood was collected from the post occipital sinus (Campbell, 1996), on the dorsal midline and just caudal to the base of the head. A 21 G or 23 G needle and a three, five or 10 ml syringe was used, depending on the size of the crocodile, and the blood transferred directly into a lithium heparin blood tube. Blood smears were made from whole blood using the cover slip method (Jain, 1986). Following blood collection the crocodile was measured: total length (TL) and snout-to-vent length (SVL) were recorded using a flexible measuring tape (±1 mm), and weighed using a harness around the forelimbs and a Pesola spring balance. Each crocodile was sexed by cloacal examination of the cliteropenis (Hutton, 1987; Leslie, 1997), and examined for clinical abnormalities including bite wounds, skin lesions, conjunctivitis, joint swelling and poor condition.

**Sample processing**

On return to the field laboratory 1.0 ml of blood was transferred to an epindorf tube for haematological analysis. The remaining blood was centrifuged using a manual desktop centrifuge, and plasma frozen for serology. If the volume of the blood sample was small it was allocated for either *Hepatozoon* examination or serology, but not both.

Thirty eight samples were examined for blood parasites and underwent heamatological analysis. Blood smears were stained with Diff-Quick Stain (American Scientific Products, Illinois, USA), (Campbell, 1995). The presence of *Hepatozoon* gametocytes was determined by examination of the Diff-Quick stained blood smears. The degree of erythrocyte regeneration was estimated by examining the red cell series, and scoring each slide on a scale of 1 to 4. On this scale, a score of 2 represents normal erythrocyte regeneration with 10-20 % orthochromic erythroblasts but no earlier stages. A score of 2.5 represents a moderate increase in erythrocyte regeneration, with late polychromatic erythroblasts through to mature and aging erythrocytes, whilst a score of 3 represents strong regeneration with basophilic erythroblasts and early polychromatic erythroblasts through mature and aging erythrocytes, but not proerythroblasts. Finally, a score of 4 would include all the other stages plus proerythroblasts.

Packed cell volumes (PCV) were determined using a Statspin MP microhaematocrit centrifuge: blood was drawn into standard microhaematocrit tubes and spun for five minutes at 12 000 G.

Total red cell counts (RCC) were performed both manually and automatically using an electronic particle counter. The automated counts were made using a Beckman Coulter Ac*T Series haematology analyzer (Coulter SA). The manual counts were made using Natt and Herrick's solution. A 1:200 dilution was made by drawing blood up to the 0.5 mark on a red blood cell diluting pipette, then filling the pipette to the 101 mark with Natt and Herrick's solution (Campbell, 1996). The diluted blood was then used to charge both counting chambers of an improved Neubauer haemocytometer (Hawksley and Sons, Lancing, UK). After 5 minutes in a damp chamber the red cells were counted in the 4 corner cells and central cell of the central large square of the counting chamber. This was repeated on the second chamber, and the average multiplied by 10 000 to obtain the total red cell count per microliter.

Haemoglobin concentrations (Hb) were determined using a Beckman Coulter Ac*T Series haematology analyzer (Coulter SA).

Red blood cell indices were calculated using standard formulas (Jain, 1986):

Mean cell volume: MCV (fl) = PCV/RCC

Mean cell haemoglobin: MCH (pg) = Hb (g/dl) x 10 / RCC

Mean cell haemoglobin concentration: MCHC (g/dl) = Hb (g/dl) / PCV

Total white cell counts (WBC) were obtained indirectly using the Unopette 5877 system (Becton-Dickinson, USA). The Unopette pipette was filled with blood (25 µl) and mixed with the phloxine B diluent in the reservoir. From this, both counting chambers of an improved Neubauer haemocytometer were charged. After 5 minutes in a damp chamber all the pink staining granulocytes were counted in both chambers.

Differential counts were done on the Diff-Quick stained smears. The percentage of heterophils and eosinophils was calculated and used to calculate total WBC (Campbell, 1996):

$$\text{Total WBC/µl} = \frac{\text{stained cells counted in chambers x 1.1 x 16 x 100}}{\text{percentage heterophils and eosinophils.}}$$

The separated plasma was stored at -10 °C in a domestic gas freezer for up to one month. After return from the research site, 30 samples were submitted to the laboratory[5] for serological testing for mycoplasmosis, using a plate agglutination test.

**Statistical analysis**

Haematological values were analyzed for significant differences ($p < 0.05$) between *Hepatozoon* -infected and -uninfected crocodiles by one way analysis of variance (ANOVA). The residuals were checked for normality of distribution with normal probability plots. Where data was not normally distributed, significance was tested by means of a Mann-Whitney test.

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**Results**

The crocodiles ranged in TL from 340 mm – 4630 mm, with a mean of 597.0 mm, and ranged in SVL from 255 mm – 1015 mm with a mean of 284 mm.

Of the 144 crocodiles caught and examined, none were visibly sick or displayed any clinical signs of disease. The body condition of all the crocodiles was good. The only external lesions observed were old healed bite injuries in one case, and a recent puncture injury in another case. Eleven crocodiles were infested with the leech *Placobdelloides multistriatus*, a prevelance of 7.6 %. One crocodile had seven leeches, and another had two. The remaining nine parasitized crocodiles each had a single leech.

No antibodies to *Mycoplasma crocodyli* were detected in any of the crocodiles tested.

*Hepatozoon pettiti* parasites were present in 21 out of 38 blood smears examined (55.3 %). The mean SVL of the 38 crocodiles tested was 426 mm. The mean SVL of the infected crocodiles was 463 mm compared to 382 mm for the uninfected crocodiles.

Six out of eight females (75 %) and 13 out of 27 males (48 %) were infected. The sex of two crocodiles was undetermined.

Two (9.5 %) of the infected crocodiles had leech infestation, as did four (23.5%) of the *H. pettiti* negative crocodiles.

Of the 21 infected crocodiles, 10 (47.6 %) showed an increased rate of red cell regeneration, with a mean score of 2.4 on our erythrocyte regeneration index. In contrast, 6 of 17 (35.3 %) uninfected crocodiles displayed an increased rate of red cell regeneration, with a mean score of 2.3 on our erythrocyte regeneration index. This was not a significant difference.

There was no significant difference between any of the haematological values of the infected and uninfected crocodiles (Table 4.1). The mean PCV and RCC of the infected group were 18.2 % and 0.62 x $10^6$ /µl respectively, compared to 17.6 % and 0.56 x $10^6$ /µl in the uninfected group.

Table 4.1: Haematological parameters of *Hepatozoon* infected Nile crocodiles compared to un-infected crocodiles.

| Parameter | *Hepatozoon* infected (n=21) | | *Hepatozoon* negative (n=17) | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| SVL (mm) | 462.6 | 223.9 | 381.7 | 123.1 |
| Mass (g) | 3804.3 | 6390.1 | 1736.5 | 2651.3 |
| PCV (%) | 18.2 | 2.0 | 17.6 | 1.9 |
| RCC (x $10^6$/µl) | 0.62 | 0.14 | 0.56 | 0.08 |
| Hb (g/dl) | 7.27 | 1.11 | 6.90 | 0.82 |
| MCV (fl) | 305.4 | 70.6 | 320.7 | 46.0 |
| MCH (pg) | 121.5 | 31.8 | 125.2 | 13.7 |
| MCHC (g/dl) | 39.7 | 2.7 | 39.4 | 3.9 |
| Eryth. regener. index | 2.4 | 0.4 | 2.3 | 0.4 |
| WBC (x $10^3$/µl) | 11.62 | 5.50 | 10.87 | 3.71 |
| Heterophils % | 19.8 | 8.7 | 21.5 | 8.9 |
| Lymphocytes % | 62.0 | 9.8 | 62.0 | 13.2 |
| Monocytes % | 1.0 | 2.2 | 0.6 | 1.1 |
| Eosinophils% | 6.0 | 5.1 | 3.5 | 4.1 |
| Basophils % | 5.7 | 4.4 | 6.2 | 4.7 |
| Azurophils % | 5.3 | 4.3 | 4.8 | 4.9 |
| Heterophils (x $10^3$/µl) | 2.00 | 0.62 | 2.19 | 0.89 |
| Lymphocytes (x $10^3$/µl) | 7.43 | 4.10 | 6.93 | 3.49 |
| Monocytes (x $10^3$/µl) | 0.11 | 0.22 | 0.07 | 0.11 |
| Eosinophils (x $10^3$/µl) | 0.65 | 0.58 | 0.38 | 0.51 |
| Basophils (x $10^3$/µl) | 0.70 | 0.75 | 0.66 | 0.54 |
| Azurophils (x $10^3$/µl) | 0.70 | 0.91 | 0.48 | 0.46 |

**Discussion**

Due to the limitations mentioned in the introduction it is probable that a capture and release survey does not provide an accurate reflection of disease prevalence in a crocodile population. On the other hand, a very low disease incidence in wild crocodiles is probable: stress is a very important predisposing factor to disease in crocodiles. Lance (1990) found that crocodilians respond to non-specific stress with a chronic increase in corticosterone secretion, an inhibition of growth, an inhibition of the reproductive system and a suppression of the immune system. Crocodiles living in a relatively pristine natural environment, with a low population density, will not be exposed to the stress experienced by farmed crocodiles in an artificially intensive environment. Furthermore, transmission of infectious diseases under natural conditions is usually far slower due to less host-pathogen exposure compared to an intensive situation.

At the time these samples were tested, the *M. crocodyli* plate agglutination test had just been developed. It was believed that the test could identify recently infected animals, but not old infections. Subsequent testing on a positive farm revealed that the test only identified very recent infection: only animals which still had lesions tested positive (Pickard, pers. comm.). The negative results obtained here do not, therefore, exclude less recent infection with mycoplasma. The mycoplasma status of the Okavango crocodiles therefore remains uncertain. An indirect enzyme-linked immunosorbent assay (iELISA) test for *M. crocodyli* has just been developed (Dawo and Mohan, 2007). This test has a high sensitivity and specificity (86 % and 100 % respectively) and will be ideal for further testing of the Okavango population.

The Samochima crocodile farm currently collects a maximum of 2000 eggs per nesting season from nests in the Panhandle region of the Okavango Delta. In return the farm is obliged to release five percent of juvenile crocodiles back into the wild. Testing of these juveniles should be undertaken before release, to avoid the risk of infecting the wild population.

In a study running concurrently to this one, Gomersall et al. (2006) found *H. pettiti* in 61 out of 186 Nile crocodiles (32.8 %). As in this study, they found no significant difference in the PCV of infected and uninfected groups. It is clear that there is a high prevalence of *H. pettiti* infection in the Okavango crocodile population, and that it appears not to be

pathogenic to the crocodile host. The mean SVL of our *Hepatozoon* infected crocodiles was greater than that of the uninfected group. It would appear that crocodiles do not lose the infection with increasing age. To date the intermediate host of *H. pettiti* remains unknown. We found no correlation between current leech infestation and *H. pettiti* infection.

A very high prevelance of *Hepatozoon* infection is not uncommon in other crocodile species. Khan et al. (1980) found that *Haemogregarina crocodilinorum* was widely distributed in the southern United States and was present in 77 (59 %) of 130 American alligators examined. Barnett et al. (1999) found a *Haemogregarina* sp. in all of nine wild alligators captured. They were anaemic compared to captive bred controls. It is not clear if the haemogregarine played a role in the anaemia, or if it was caused solely by leech infestation and lower nutritional plane of the wild alligators. A prevelance of 71.4 % was reported for *Hepatozoon caimani* in caimans (*Caiman crocodilus*) in Western Brazil (Viana et al., 2005), and Ladds et al. (1990) found a haemogregarine in 16 of 25 (64 %) salt- and fresh water crocodiles (*C. porosus* and *C. novaeguineae*).

Further research is required in several of the crocodilian diseases to elucidate the epidemiology of these diseases, and particularly the role of wild crocodilians as reservoirs of infection. Pox virus, adenovirus, West Nile virus, chlamydia and mycoplasmosis are of particular interest.

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# CHAPTER 5

## Conclusion

A set of reference values has been established for the haematological and blood biochemical parameters of wild Nile crocodiles in the Okavango Delta. Given the limited sample size, and the skewered sex ratio, these values may not be truly representative. Furthermore, in other reptiles "normal ranges" often vary with season, environmental conditions, nutritional status and population dynamics. Additional studies in different seasons will be required to build up a more complete picture in wild Nile crocodiles. Nevertheless, these reference ranges are the best available at this stage, and will be useful for diagnostic investigations involving clinical pathology. These values are also available as baseline data for ecological studies using the crocodile as indicator of its environment.

The comparatively low mean haematocrit and total protein values obtained from this sample of the population may indicate a poor nutritional status or a heavy parasite burden. The high prevalence of *Hepatozoon pettiti* did not have an influence on haematocrits. More research on the internal helminth parasites of Nile crocodiles, and their effect on the host, is required. To date it is believed that the ascaridoids are largely non pathogenic. The possibility of a poor nutritional status seems unlikely. The density of this population is relatively low (Bourquin, pers. comm.), and the food resource should not be a limiting factor. Furthermore, the animals appear to be in good body condition. Nevertheless, their somewhat compromised metabolic state, as indicated by the low Ht and total protein, may be a contributing factor in the apparent inability of the population to recover to previous numbers. [Between 1957 and 1969 an estimated 50 000 crocodiles were shot and trapped by hide-hunters (Pooley, 1982) while in 1998 the total crocodile population was estimated by aerial surveys flown along the river course at 10 000 animals (Simbotwe and Matlhare, 1988).]

The normal range of intestinal flora was established from a sample of wild Okavango Nile crocodiles. Bacteria were cultured from all crocodiles, with an average of 2.7 species per crocodile. Fungi were cultured from approximately half the crocodiles, with an average of 0.55 species per crocodile. Seven of the bacteria and three of the fungi isolated have

previously been associated with septicaemia / fungaemia. This supports the view that normal intestinal tract inhabitants can become pathogenic in an immuno-compromised crocodile host, and highlights the importance of optimal husbandry practices in an intensive crocodile farming environment.

Surprisingly, *Salmonella* were not found to be part of the normal intestinal flora of the wild population. In contrast *Salmonella* were cultured from the farmed hatchlings at Krokovango. This is of importance because of the intended release of farmed juveniles into the wild. Further testing of the wild population should be carried out to confirm its *Salmonella* status. If the population remains *Salmonella* negative, the intended policy of release of farmed juveniles will have to be re-evaluated.

The probiotic trial did not have the beneficial effect expected. This was surprising in light of the widespread use of probiotics in other species. There were several factors in the design of the experiment that may have influenced the results. There is no doubt that there remains a lot of potential in this field. More trials are required, possibly with changes to the method of probiotic administration, age of the hatchlings, and bacterial species included in the probiotic.

The disease survey indicated a healthy population in terms of the number of sick individuals encountered. However, this is not an accurate reflection of prevalence of disease, as discussed in Chapter 4. As more crocodile specific diagnostic tests are developed, a more complete disease survey will become possible. Further research is required to elucidate the role of wild crocodilians as reservoirs of several diseases, including pox virus, adenovirus, West Nile virus, chlamydia and mycoplasmosis.

A very high prevalence of *Hepatozoon pettiti* was established. The low pathogenicity of this parasite was confirmed by haematological analysis of infected and un-infected crocodiles. The vector of this parasite remains unknown.

Mycoplasmosis was not found to occur in the Okavango crocodiles. However, due to the low sensitivity of the plate agglutination test used, the mycoplasma status of the Okavango population remains uncertain.

Further work is essential: the management plan for the crocodiles of the Okavango Delta must include concrete recommendations regarding sustainable crocodile ranching.

Crocodile ranching has a high conservation value as it creates incentives to ensure long-term sustainable management of the wild population (MacGregor, 2002). One of the measures that can be implemented to ensure long-term sustainability is return of captive raised juveniles to the wild. This is the current policy concerning crocodile ranching in Botswana. However, the benefit of bolstering the wild population with captive juveniles will be negated if mycoplasmosis is inadvertently introduced into a naïve wild population.

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